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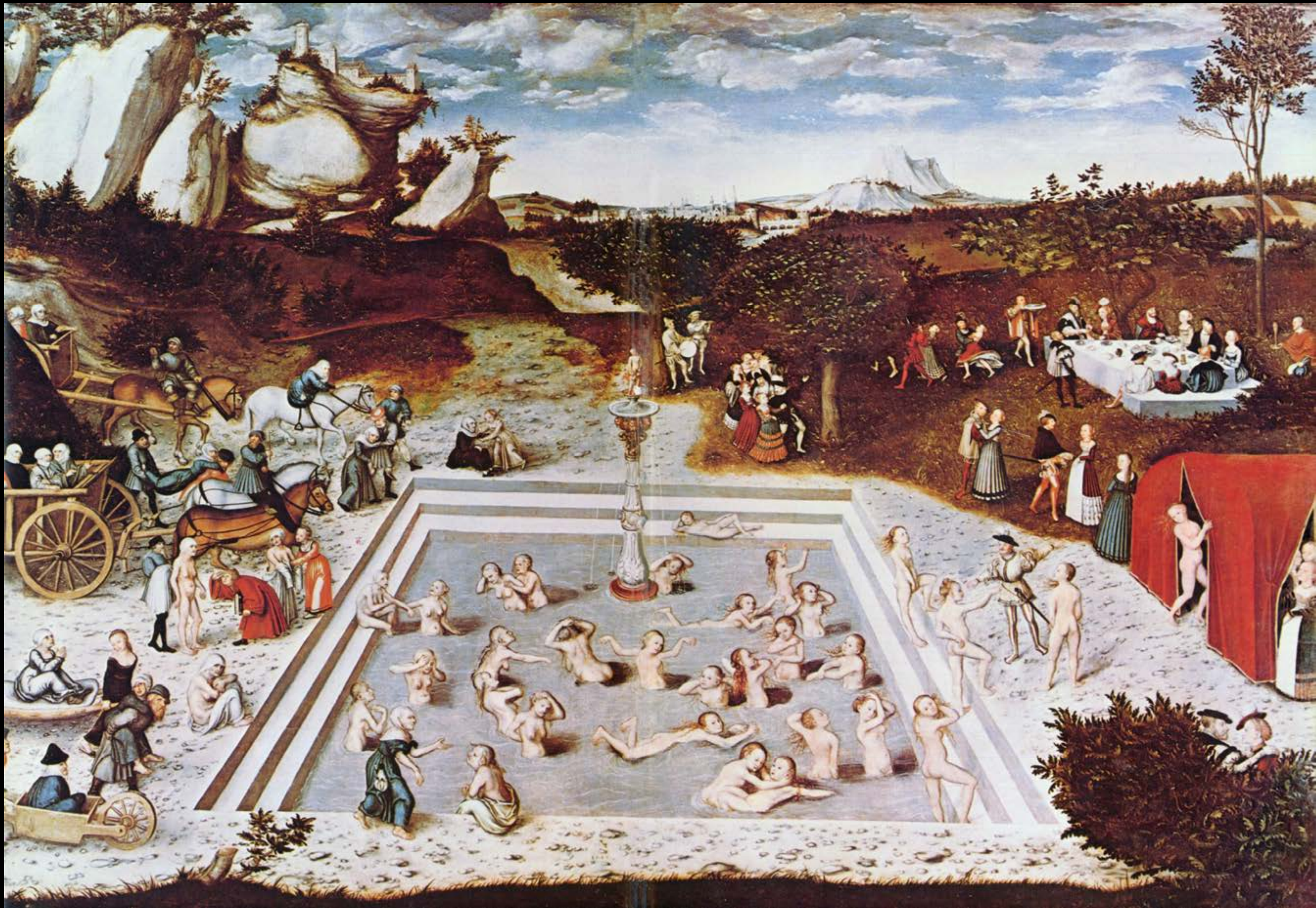
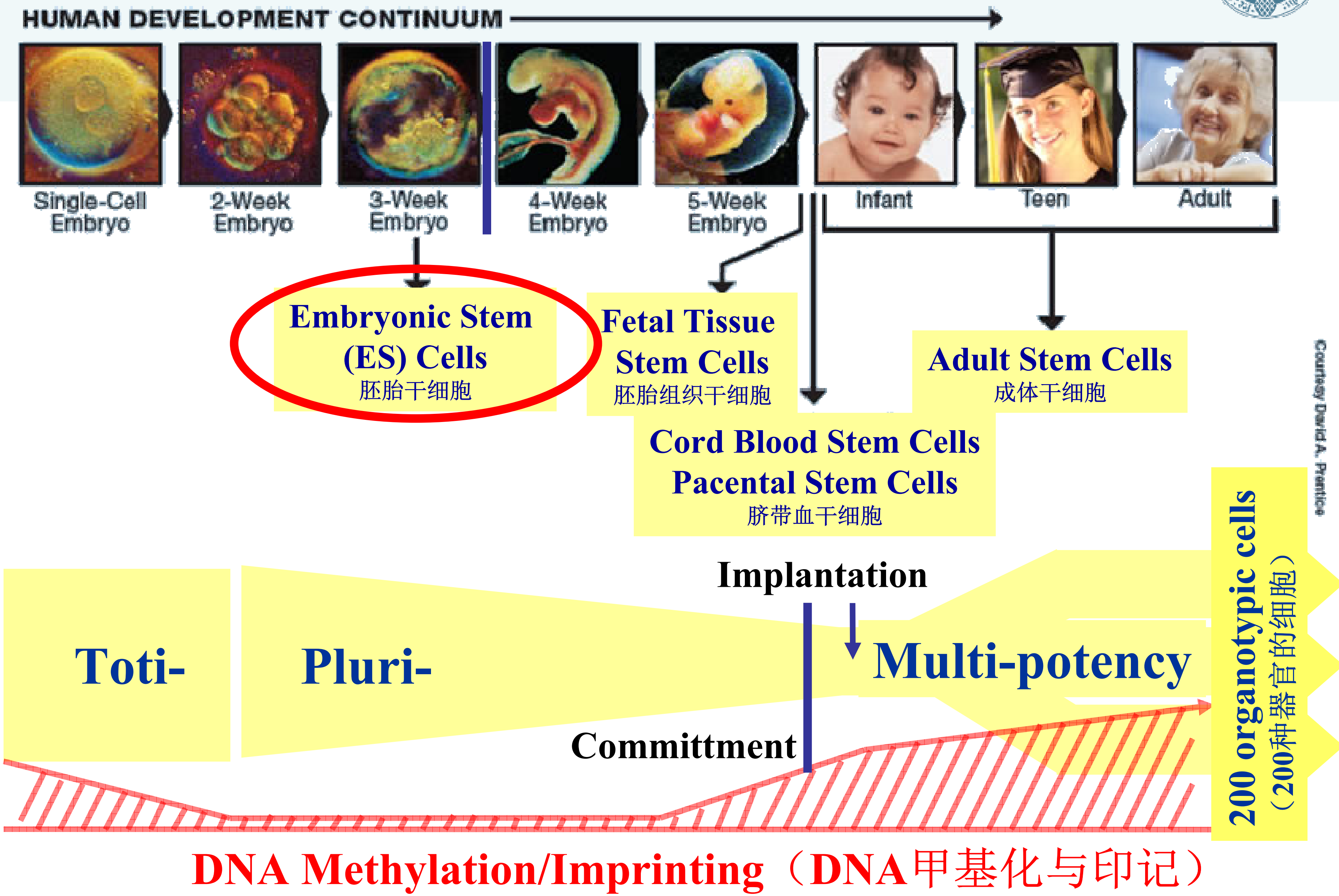
Pluripotent stem cells for research and clinical application

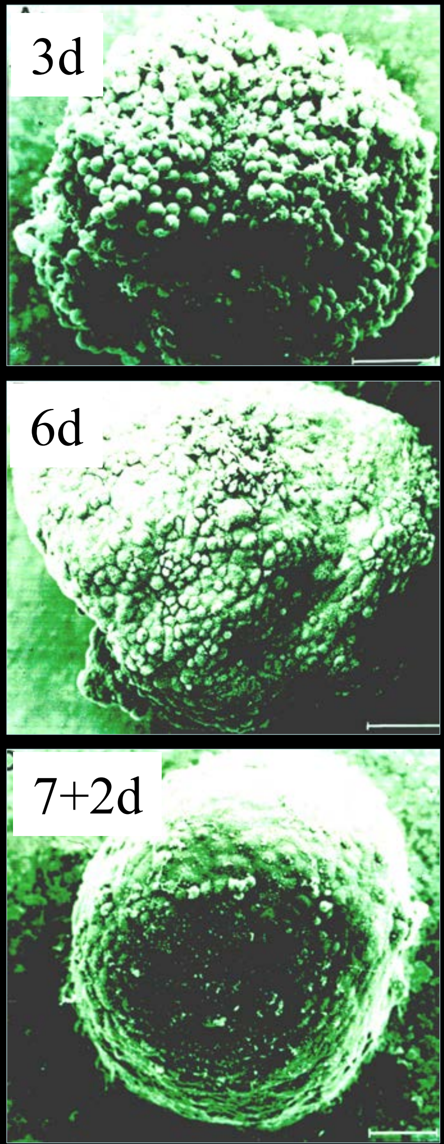
Prof. Dr. J. Hescheler
University of Cologne,
Cologne, Germany



Lucas Cranach der Ältere, 1546

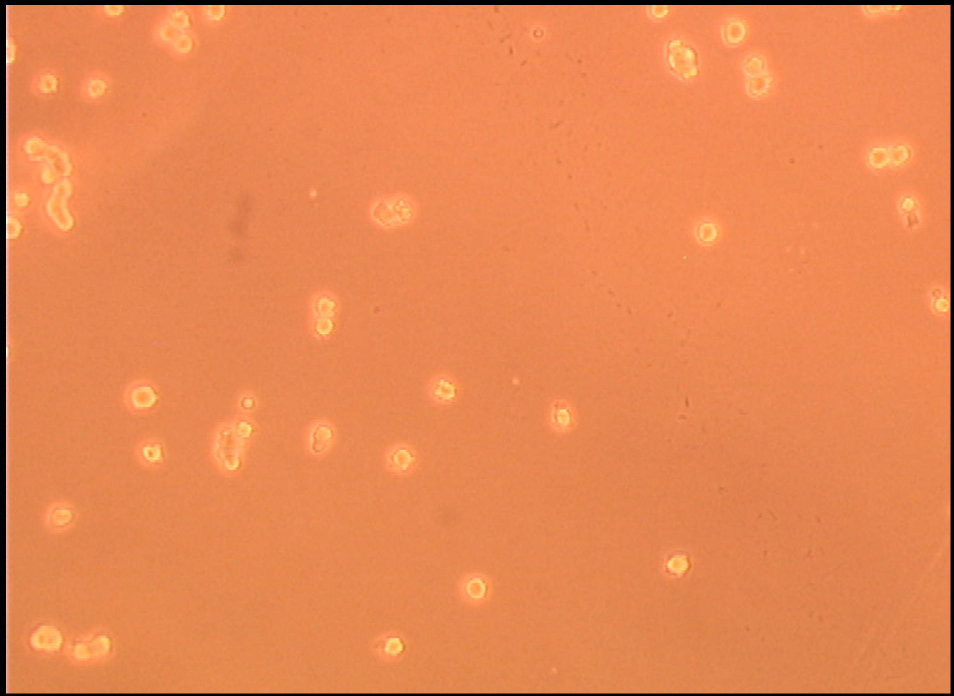
Continuity and diversity of stem cells（干细胞的连续性和多样性）





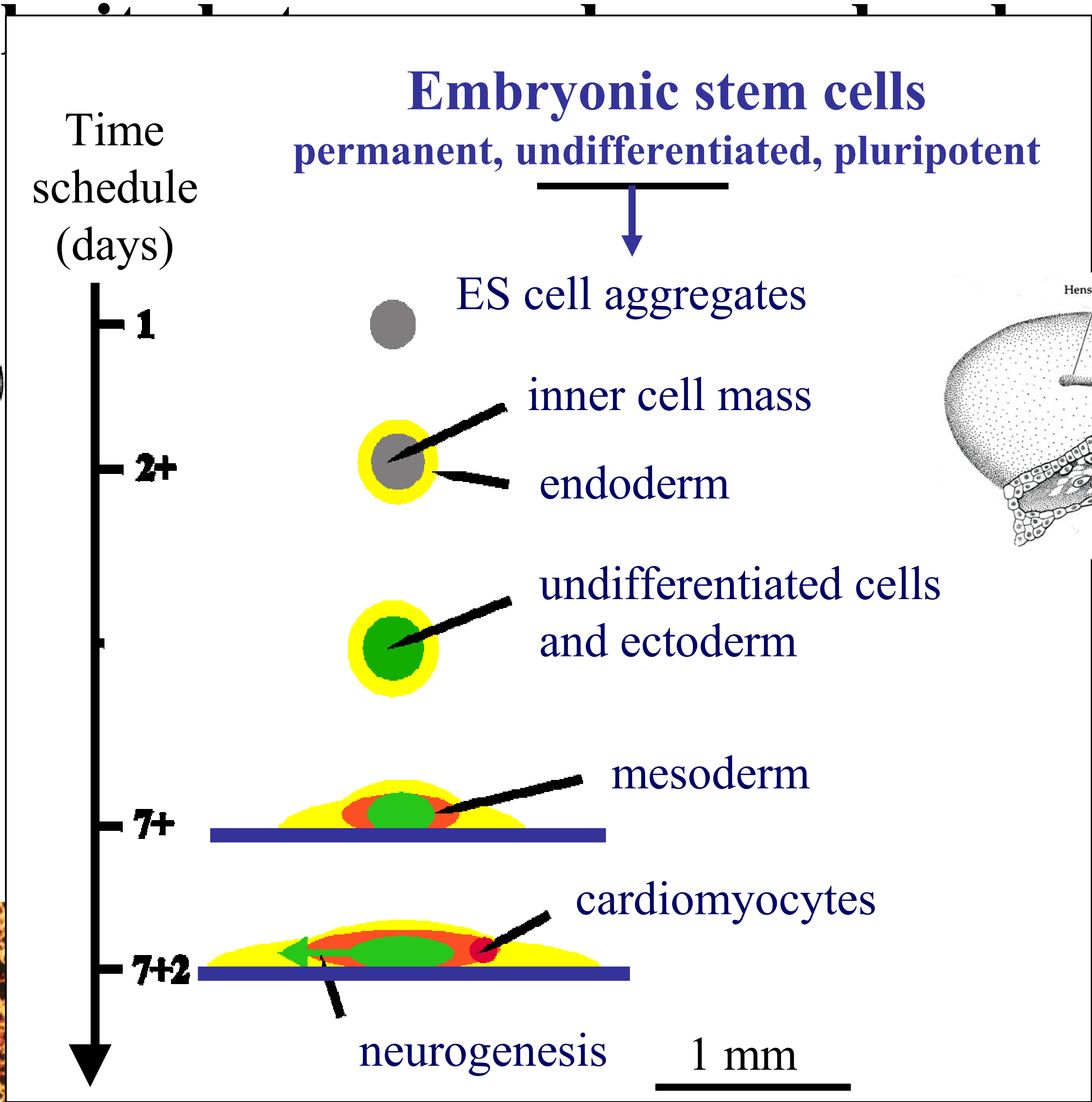
Main features of embryonic stem cells:

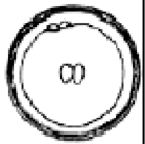
- Pluripotency
- Completely Undifferentiated (low DNA Imprinting)
- Self Renewal (Unlimited Cell Source,
  Colony Formation)



mESC line CGR8

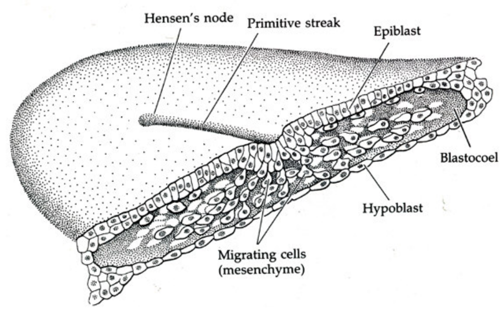
Simi… …oid body



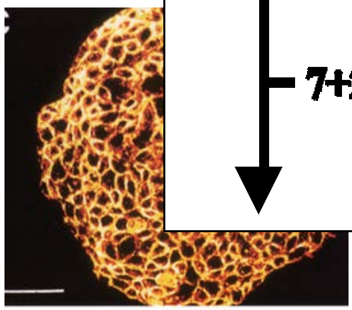


a

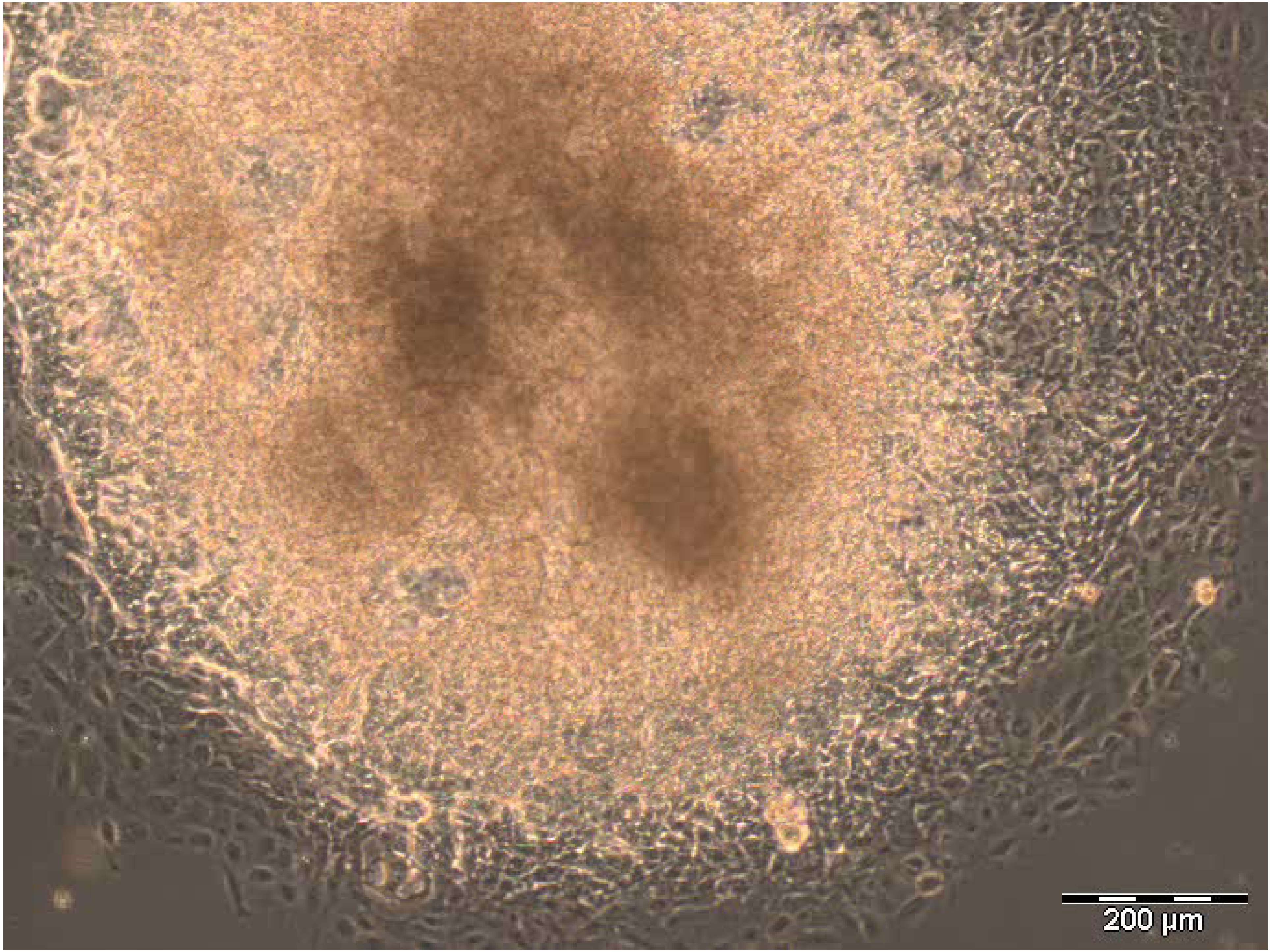
b

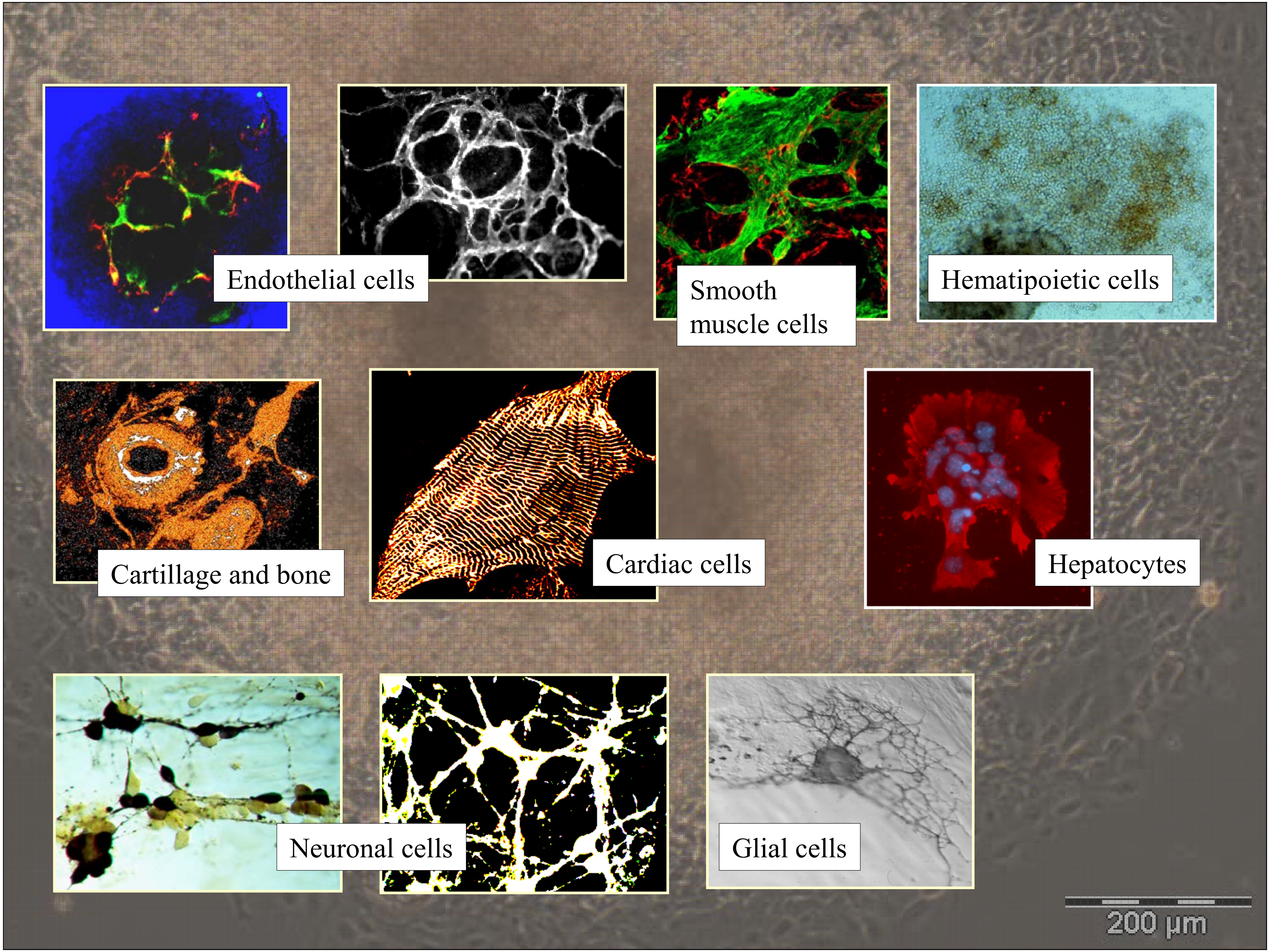


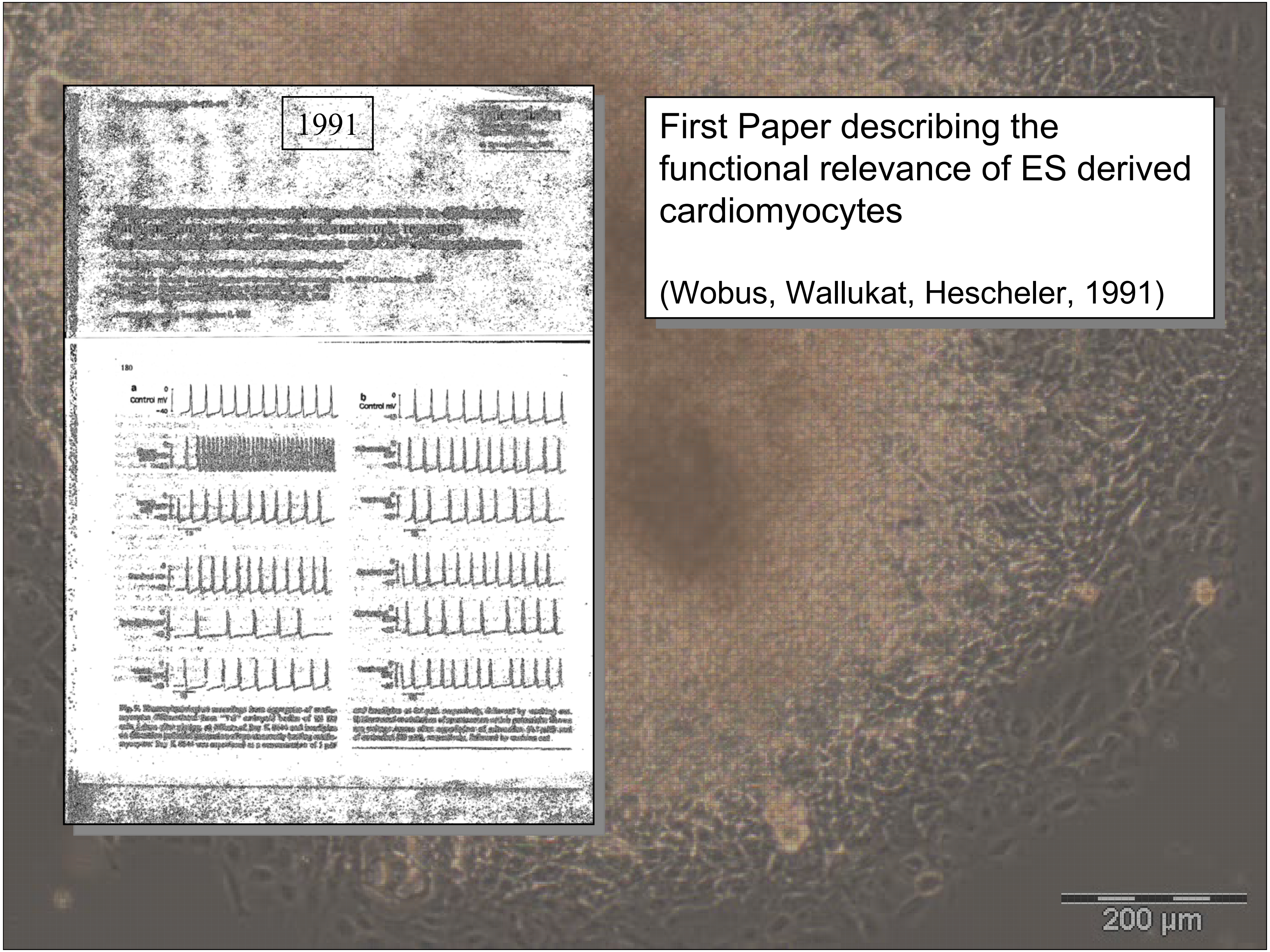
Mesoderm



Alpha-Fetoprotein


200 µm


Endothelial cells
Smooth
muscle cells
Hematipoietic cells
Cartillage and bone
Cardiac cells
Hepatocytes
Neuronal cells
Glial cells
200 µm

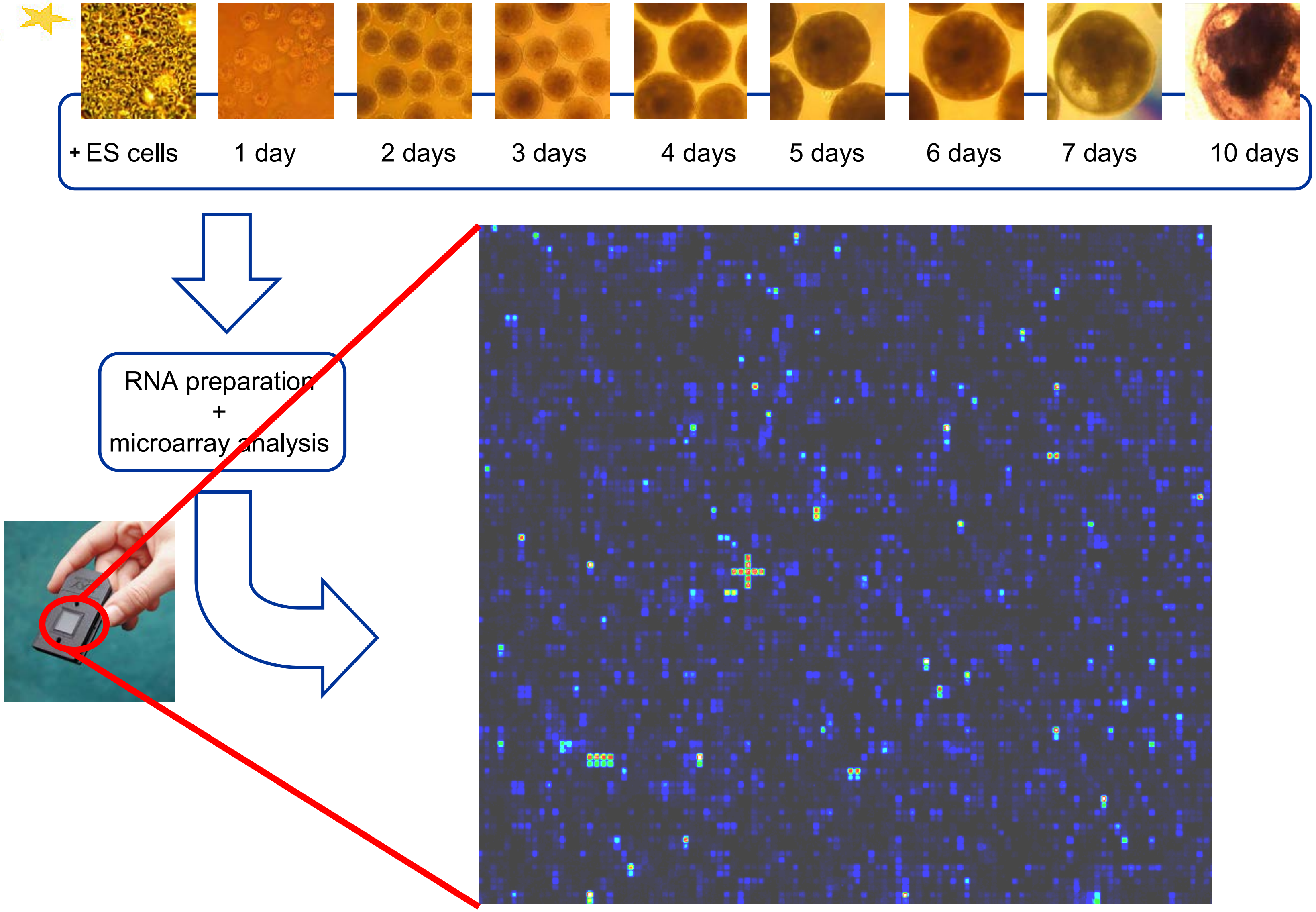


First Paper describing the functional relevance of ES derived cardiomyocytes

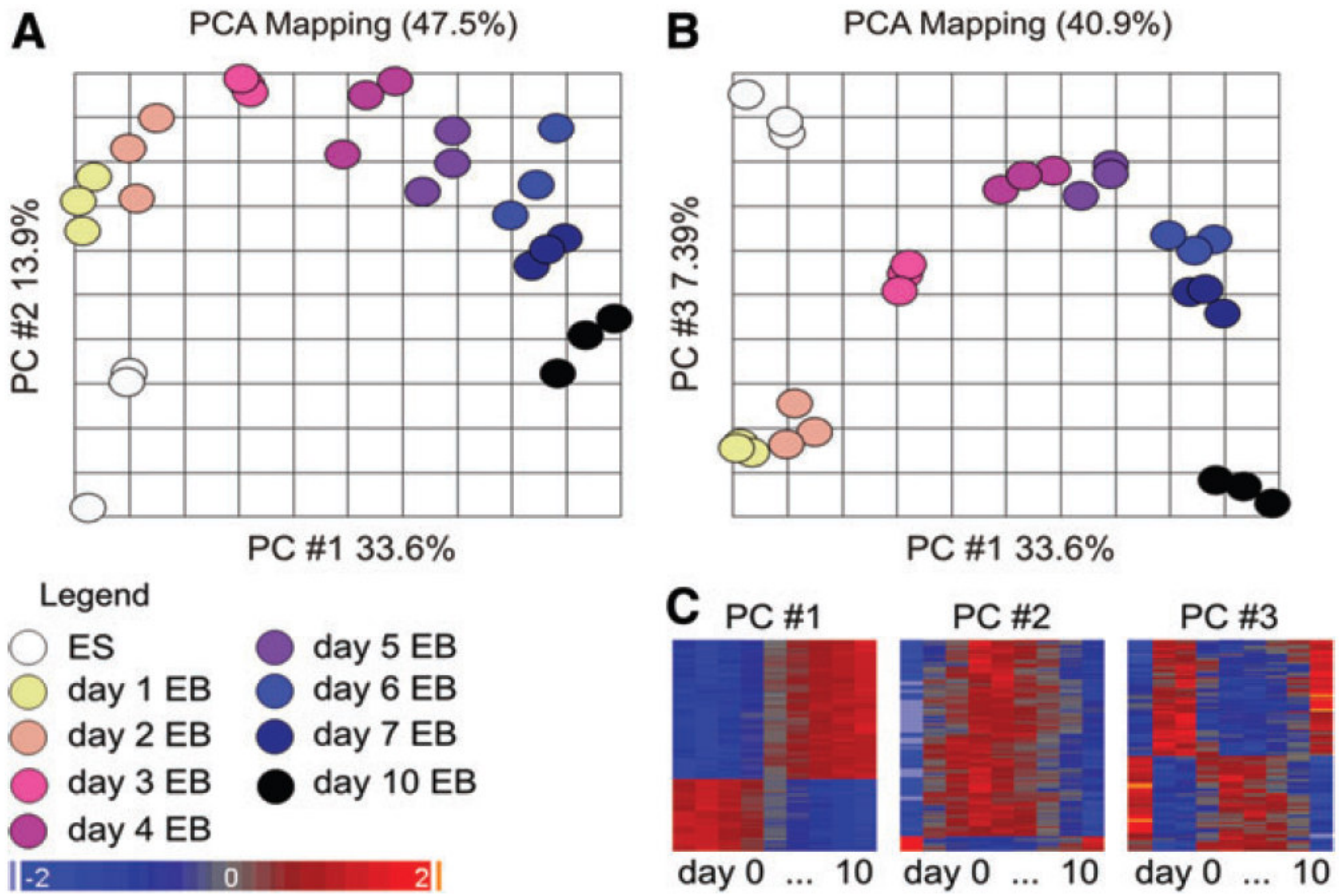
(Wobus, Wallukat, Hescheler, 1991)

Affymetrix Expression Profiling of EBs

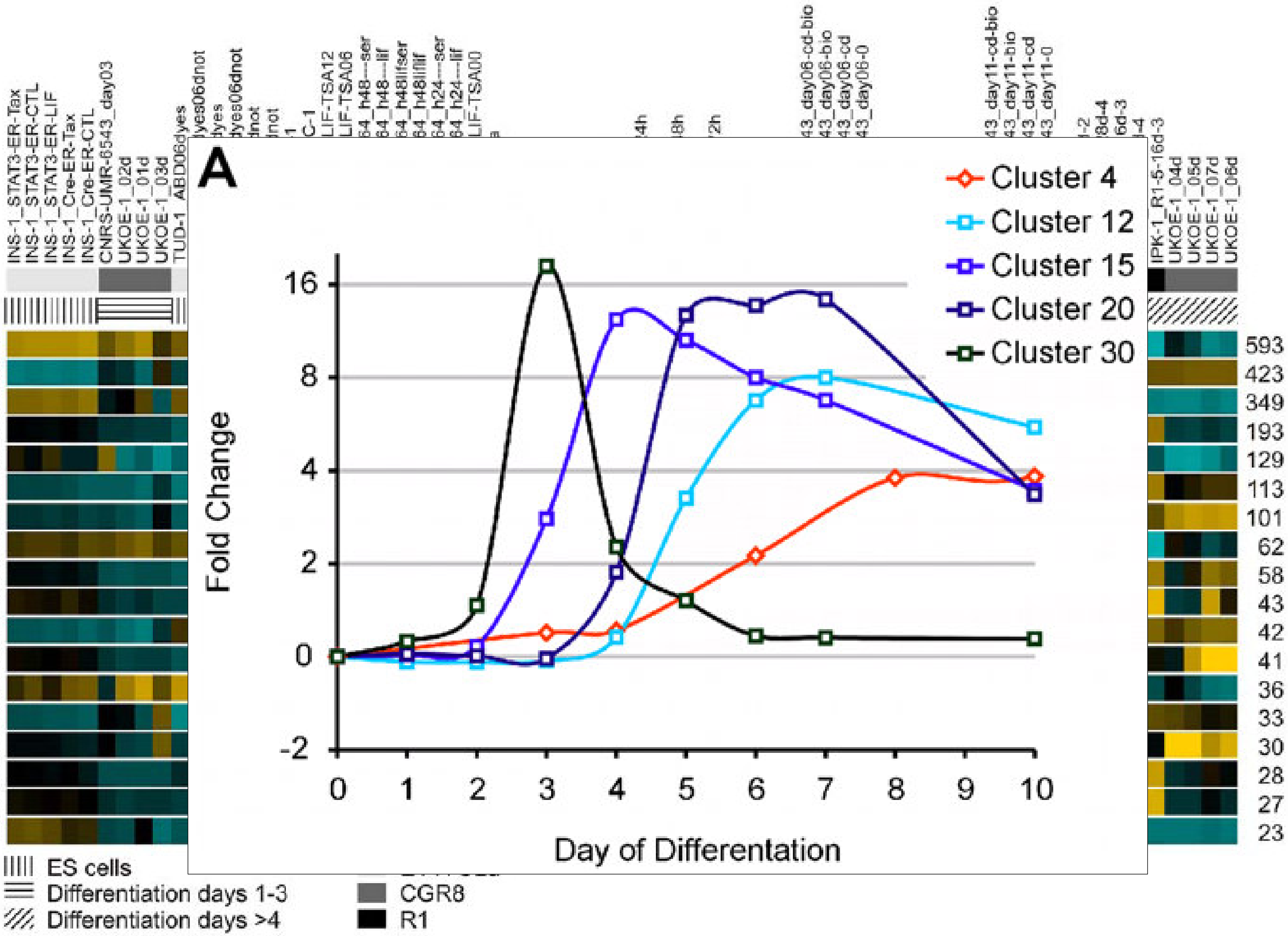
Formation of Embryoid Bodies from CGR8 cells in hanging

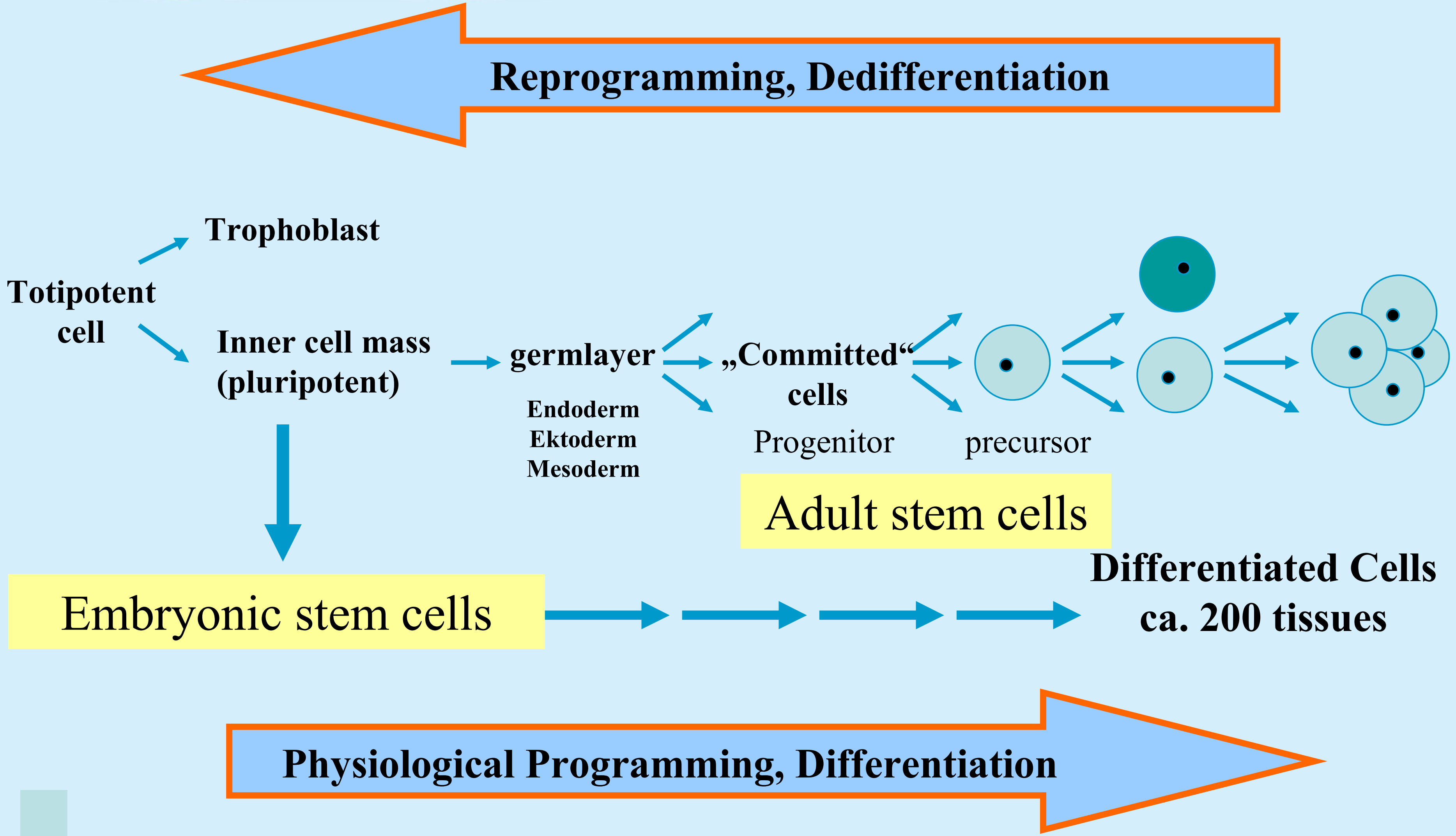


Principal component analysis (PCA) mapping

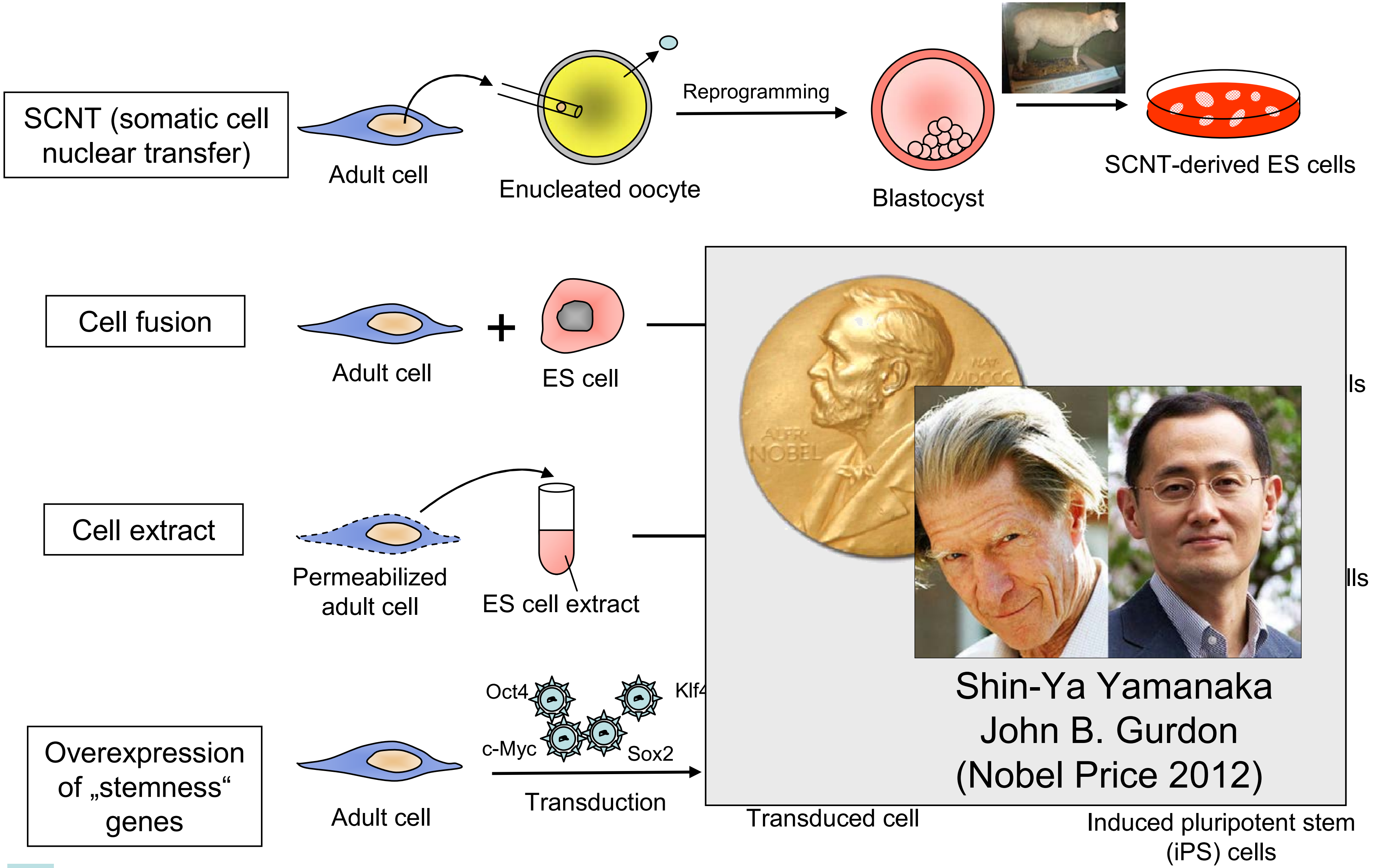


Global hierarchical clustering analysis of the FunGenES microarray data



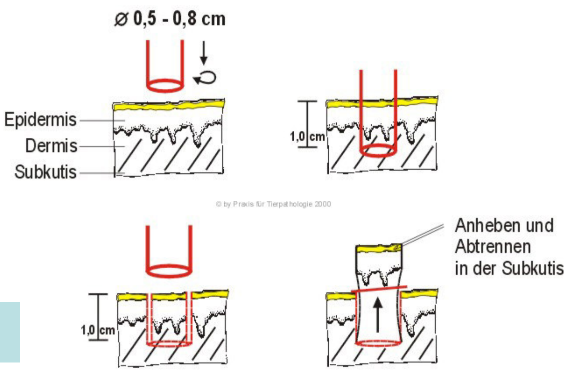

Reprogramming, Dedifferentiation
Trophoblast
Totipotent cell
Inner cell mass (pluripotent)
germlayer
Endoderm
Ektoderm
Mesoderm
„Committed“ cells
Progenitor
precursor
Adult stem cells
Embryonic stem cells
Differentiated Cells ca. 200 tissues
Physiological Programming, Differentiation

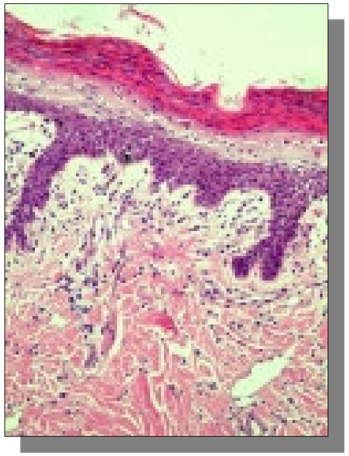
Methods to derive autologous stem cells by nuclear reprogramming



Gewinnung von Hauptbiopsien

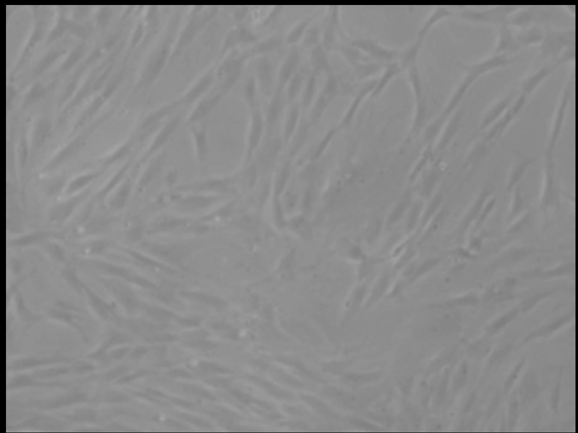
Gewinnung von Hautstanzbiopsien

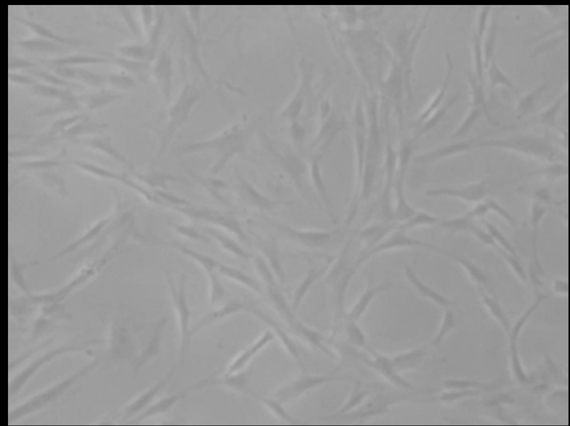


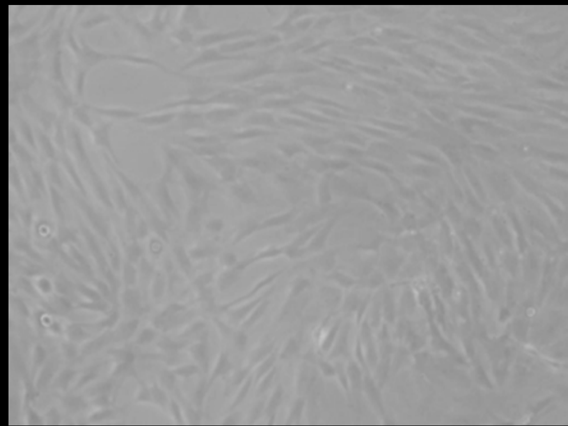


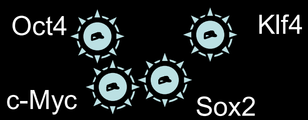
Human fibroblasts derived from a skin biopsy (JH)

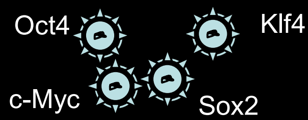
Passage 1

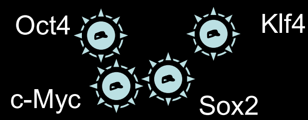






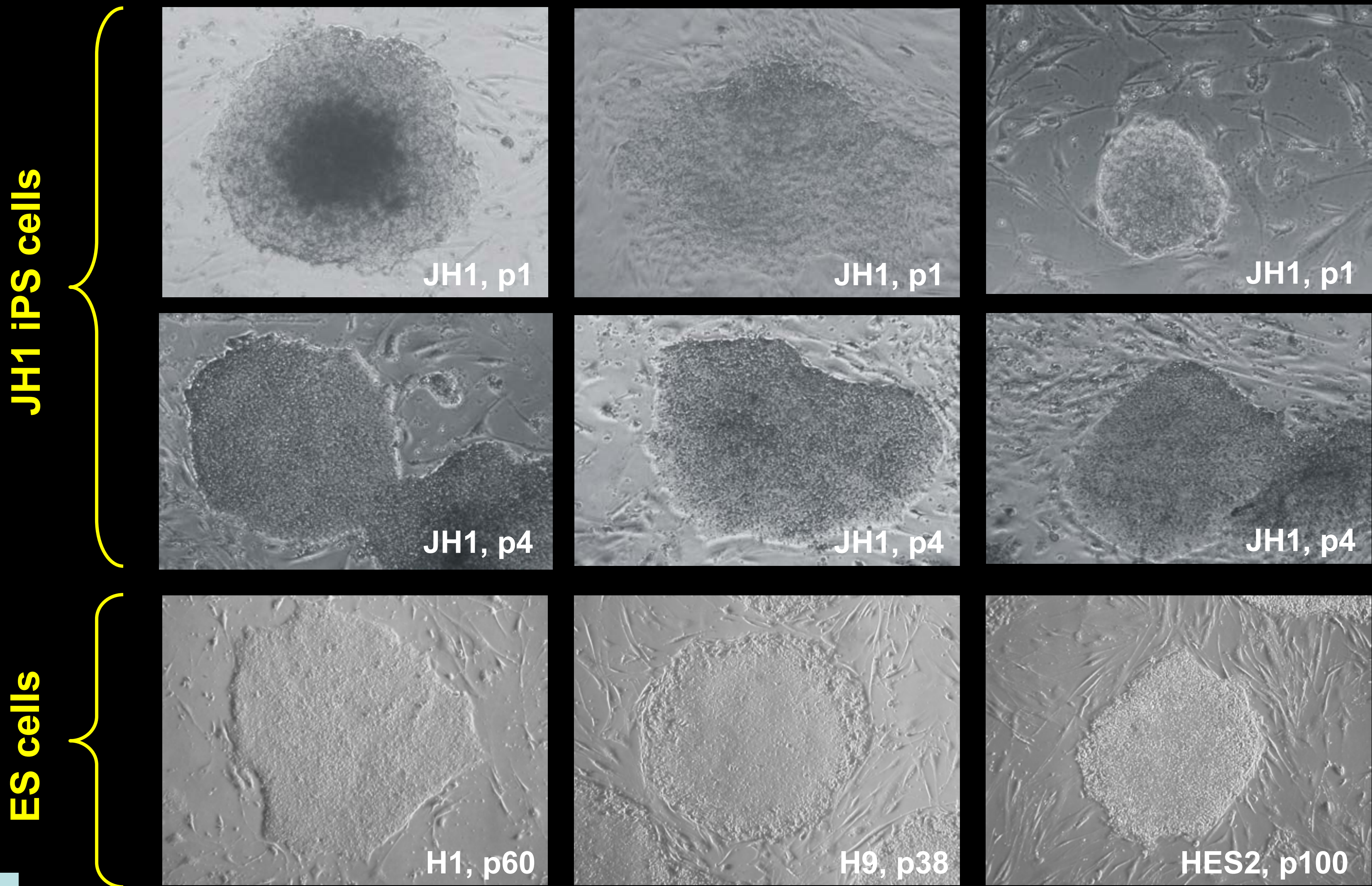






Azra Fatima (AG Tomo Saric)

Undifferentiated human ES and iPS cells

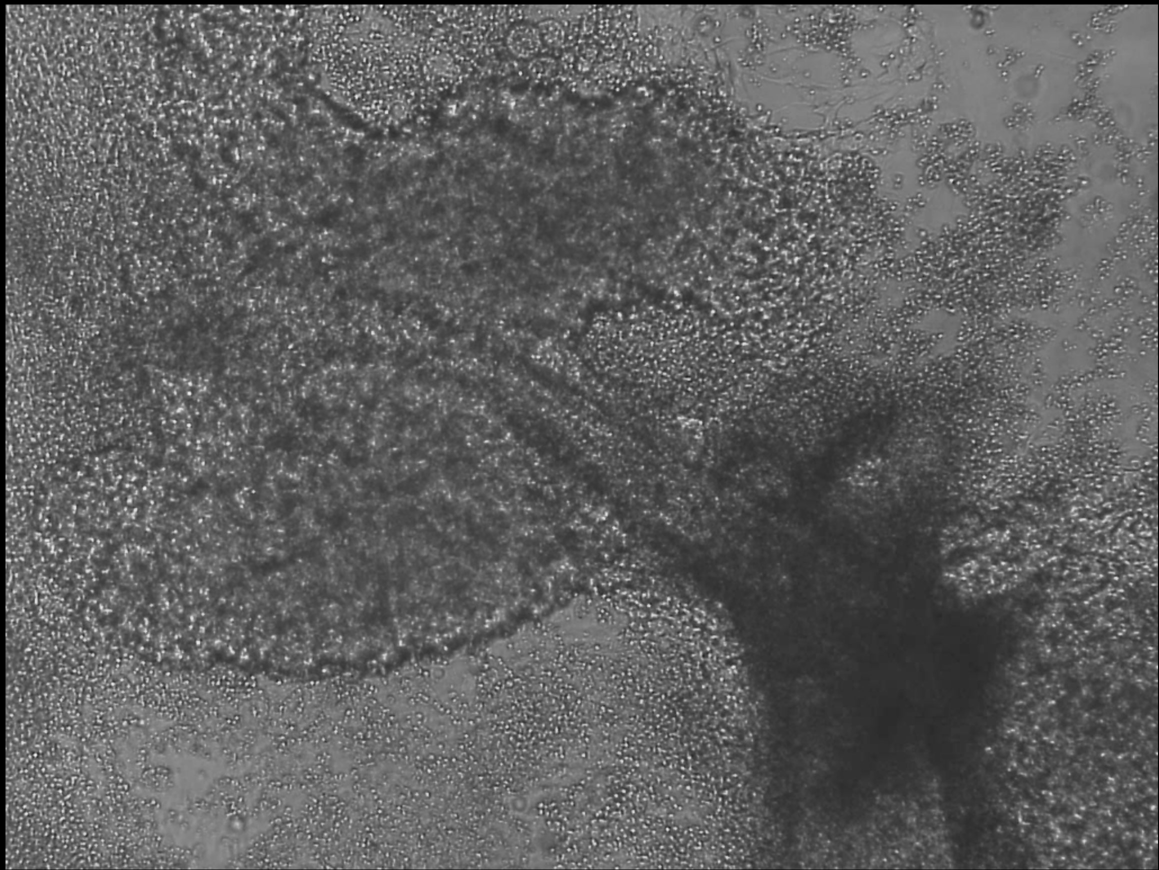


Azra Fatima/Manoj Gupta (AG Tomo Saric)

Human JH1 iPS cell colonies are positive for alkaline phosphatase

Azra Fatima (AG Tomo Saric)

Human JH1 iPS cells differentiate into beating cardiomyocytes

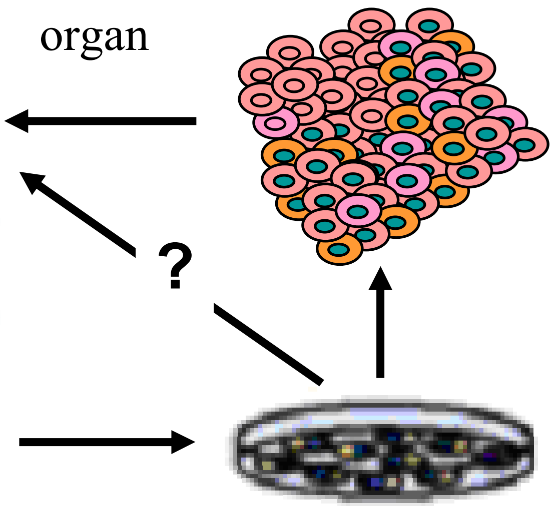


Azra Fatima (AG Tomo Saric)

Concept of Cell Transplantation in Regenerative Medicine

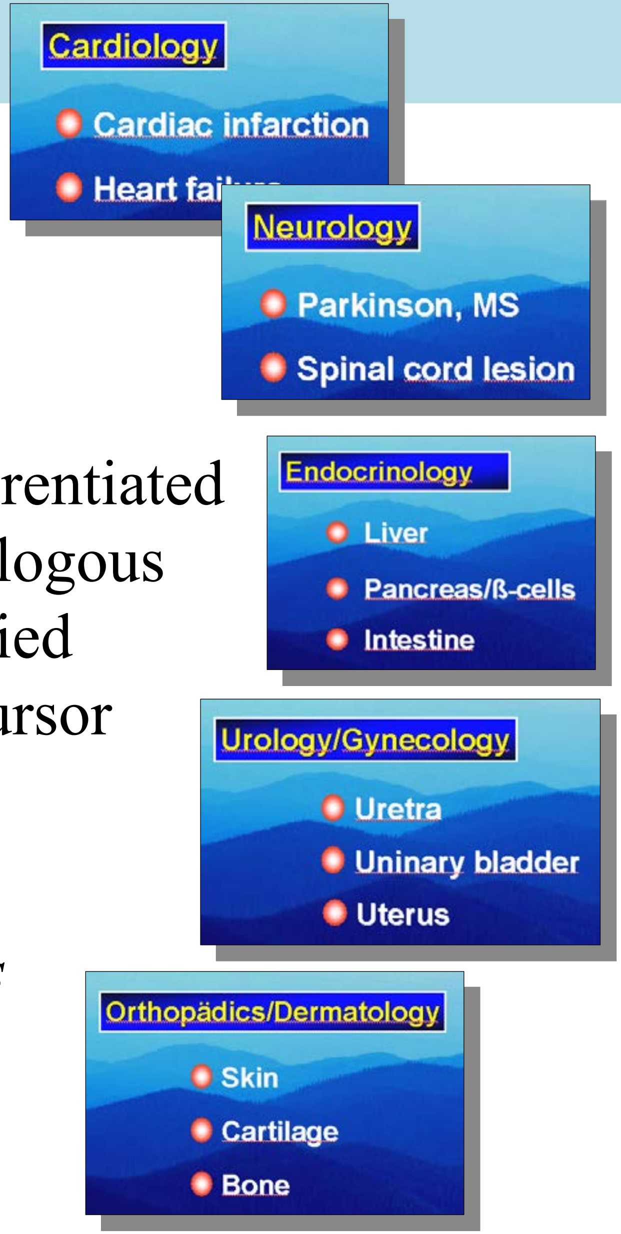
University of Cologne

Transplantation into the diseased organ

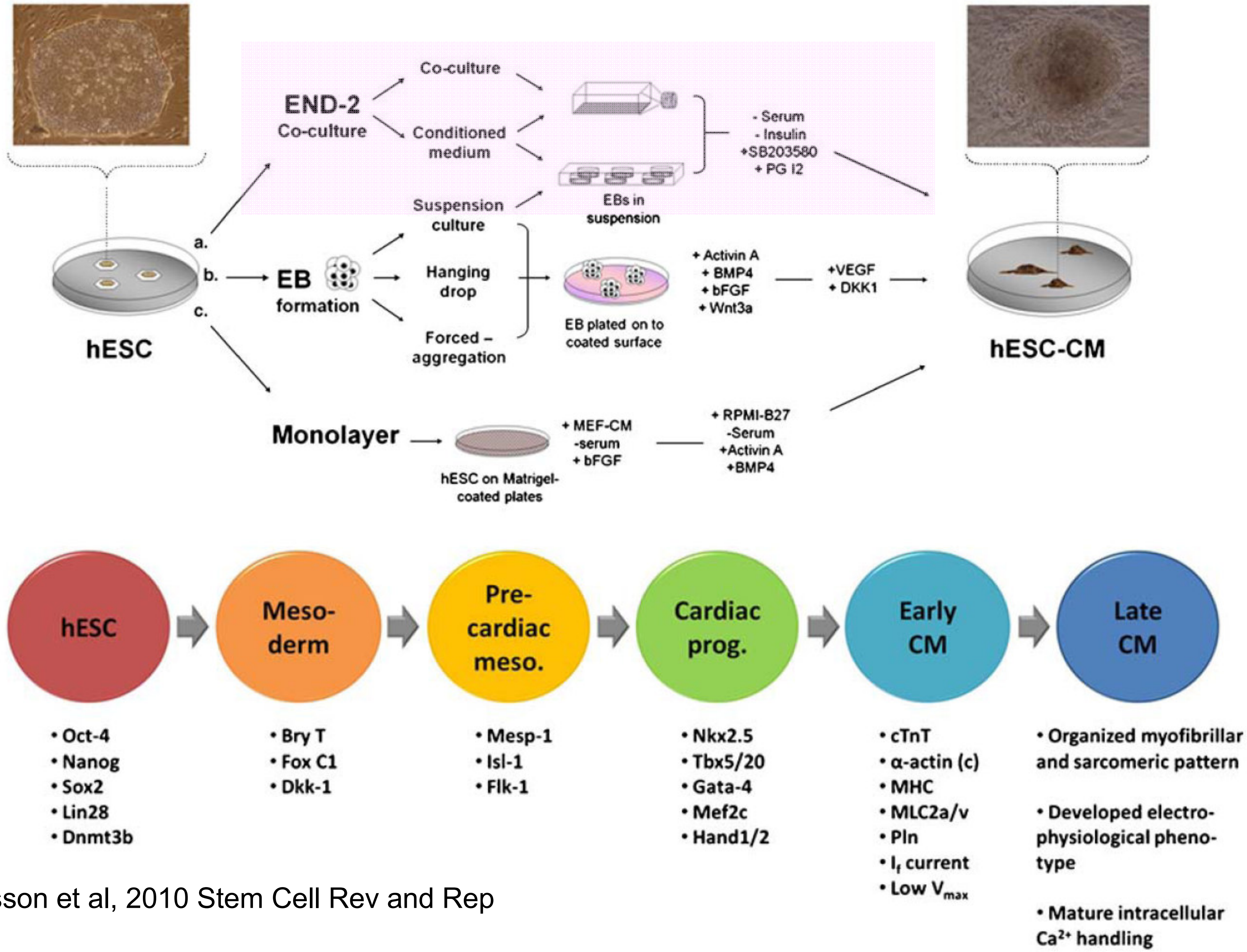


Differentiated Autologous Purified Precursor Cells

Induced Pluripotent Stem Cells



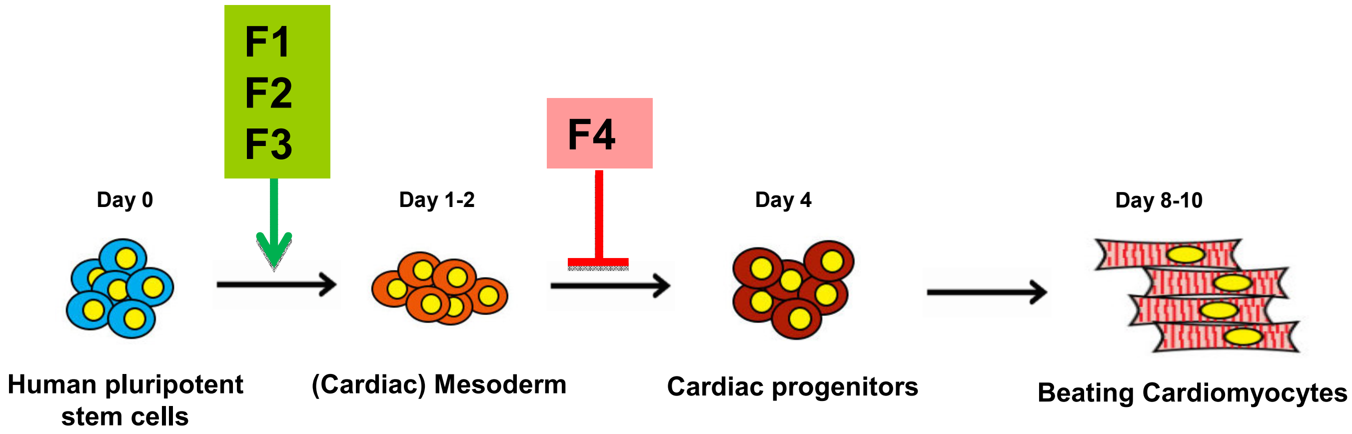
Cardiomyocyte Differentiation Strategies



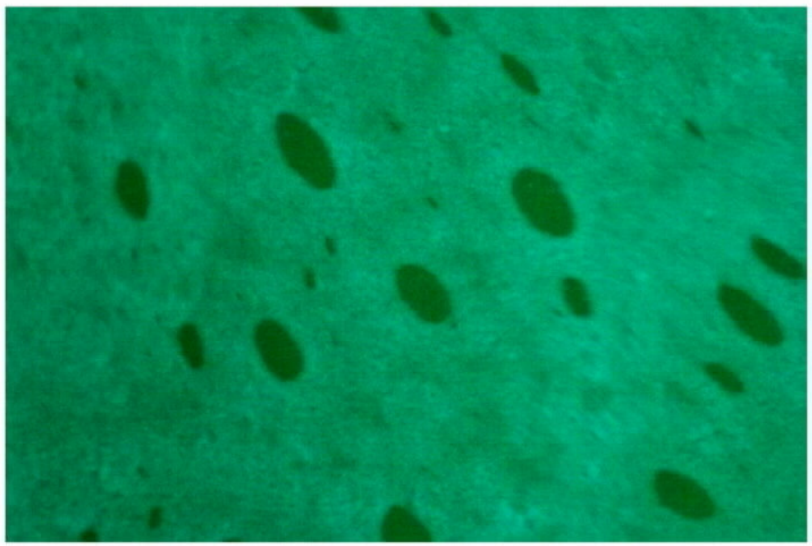
Vidarsson et al, 2010 Stem Cell Rev and Rep

Human Cardiac Differentiation 心肌分化

Dr. Stefan Frank
AG Kurian



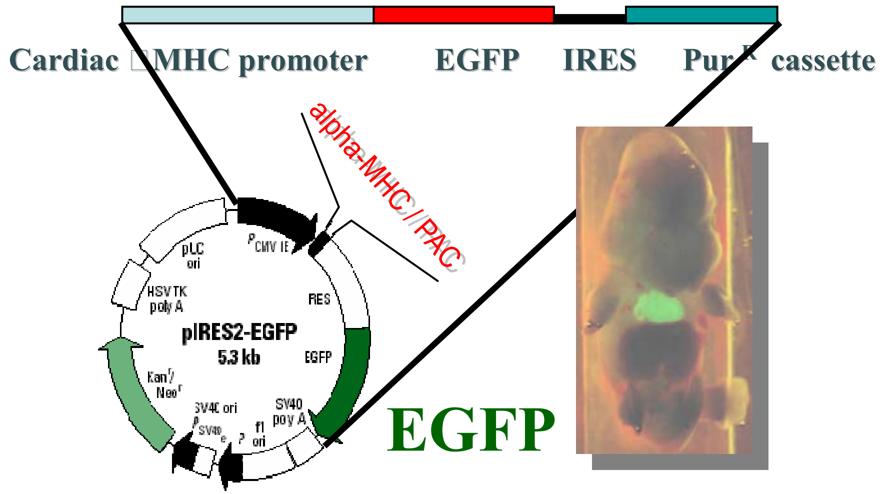
Human pluripotent stem cell derived cardiomyocyte

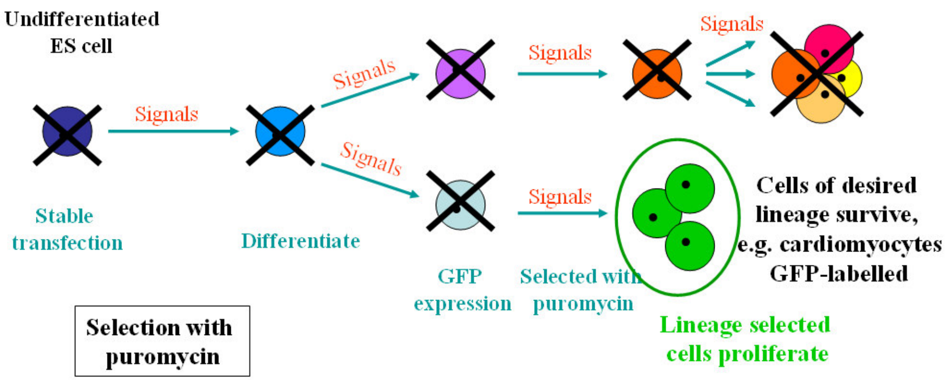


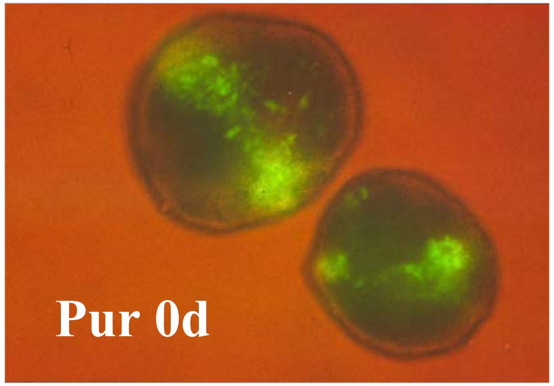
Cardiac specific reporter

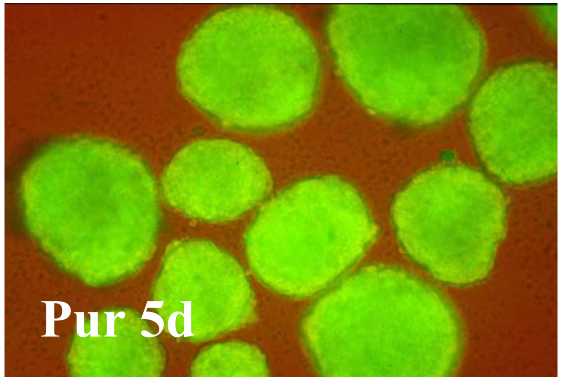
Uniform sheet of homogenous, beating cardiomyocytes after 10 days of differentiation in defined conditions

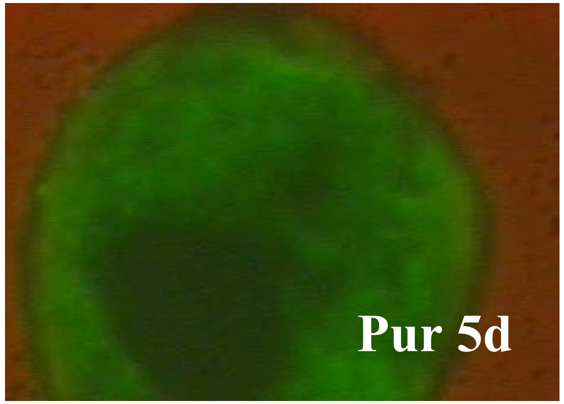
Cardiac Purification by Lineage Selection



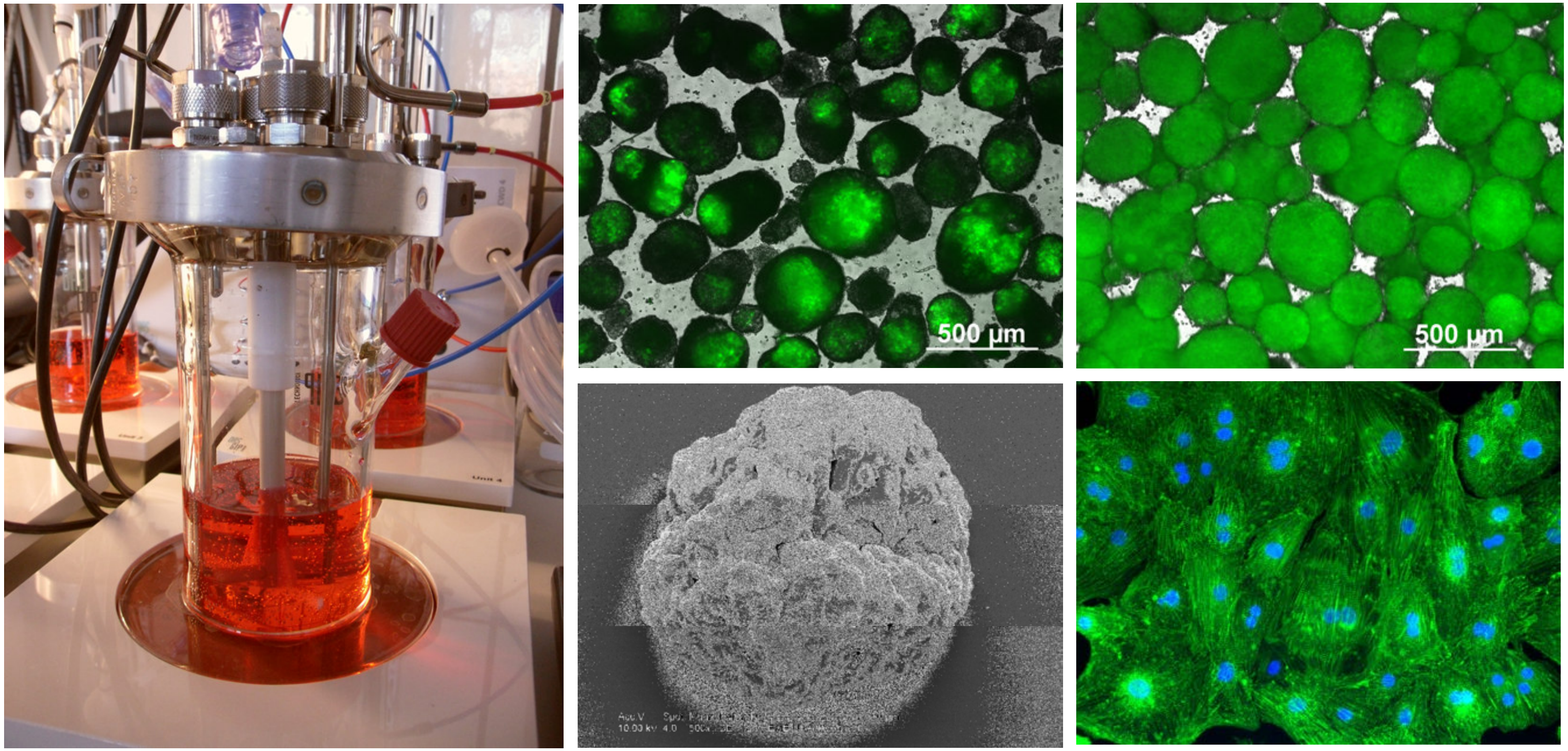








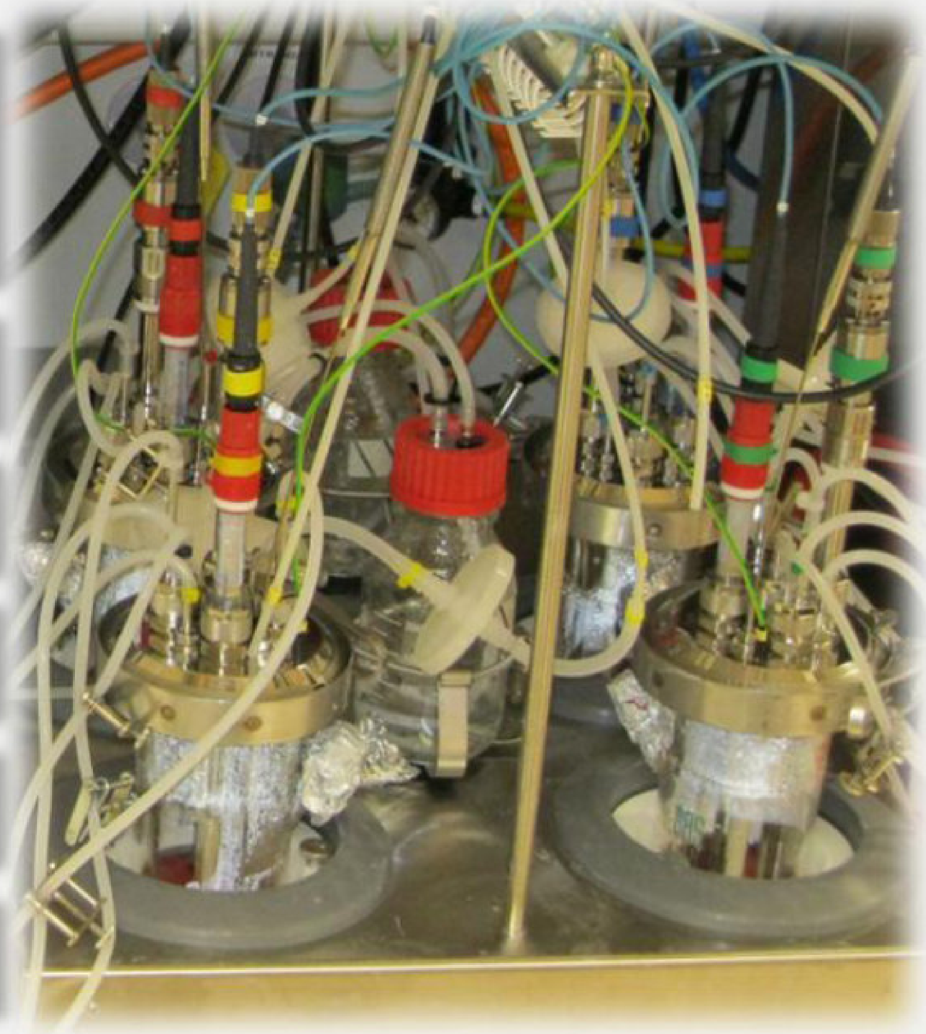
Differentiation and purification of murine iPS-CM in controlled suspension bioreactor



Karsten Burkert (Saric group)

Stirred Tank Bioreactors

- ✓ Fully controlled Environment (pO_2, pH, temperature, agitation…)
- ✓ Scalability
- ✓ Automation
- ✓ Reproducibility
- ✓ Homogeneity
- ✓ Easy sampling



Manuel Corrondo
Paula Alves
Magarida Serra

iBET
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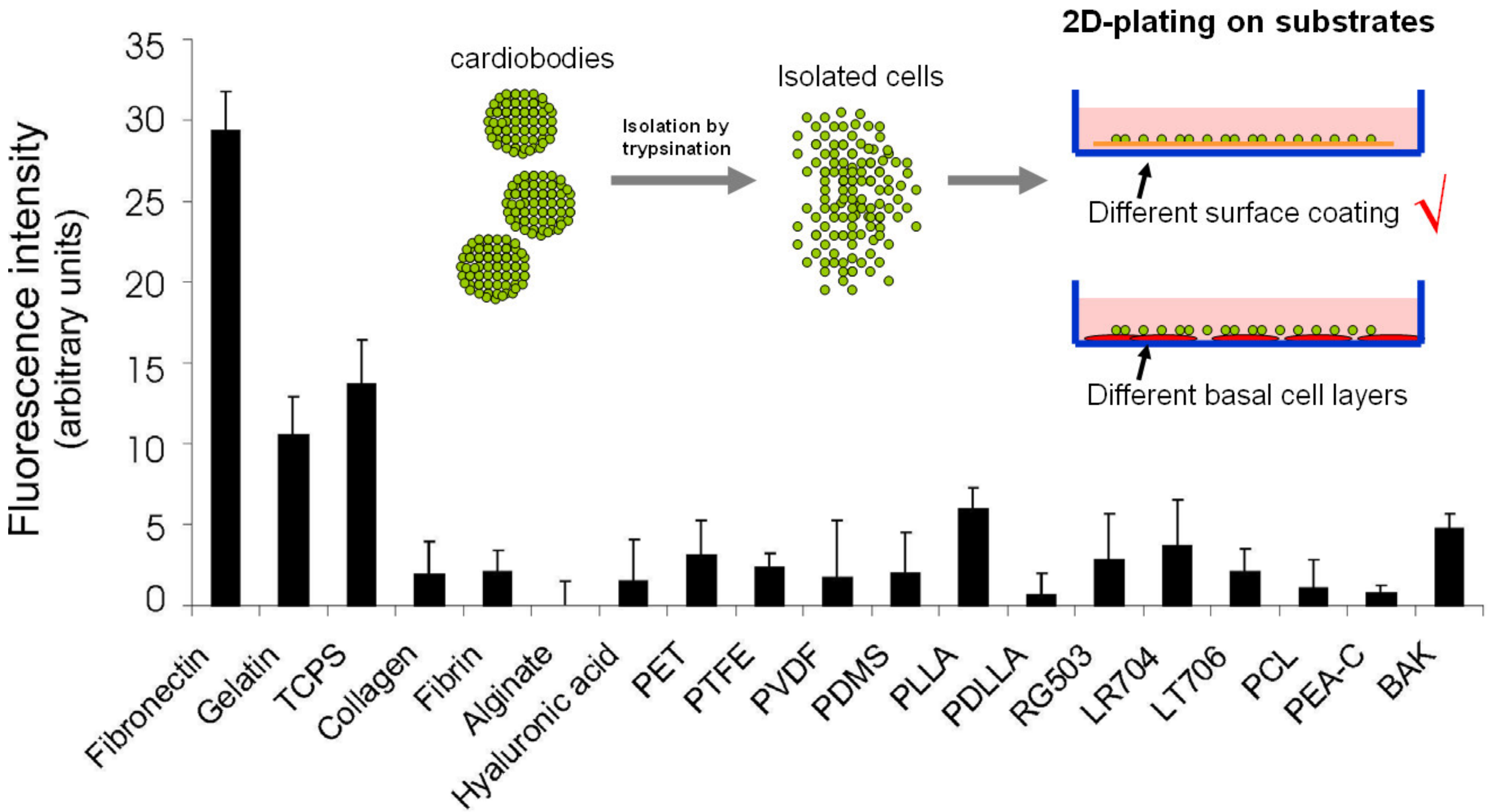
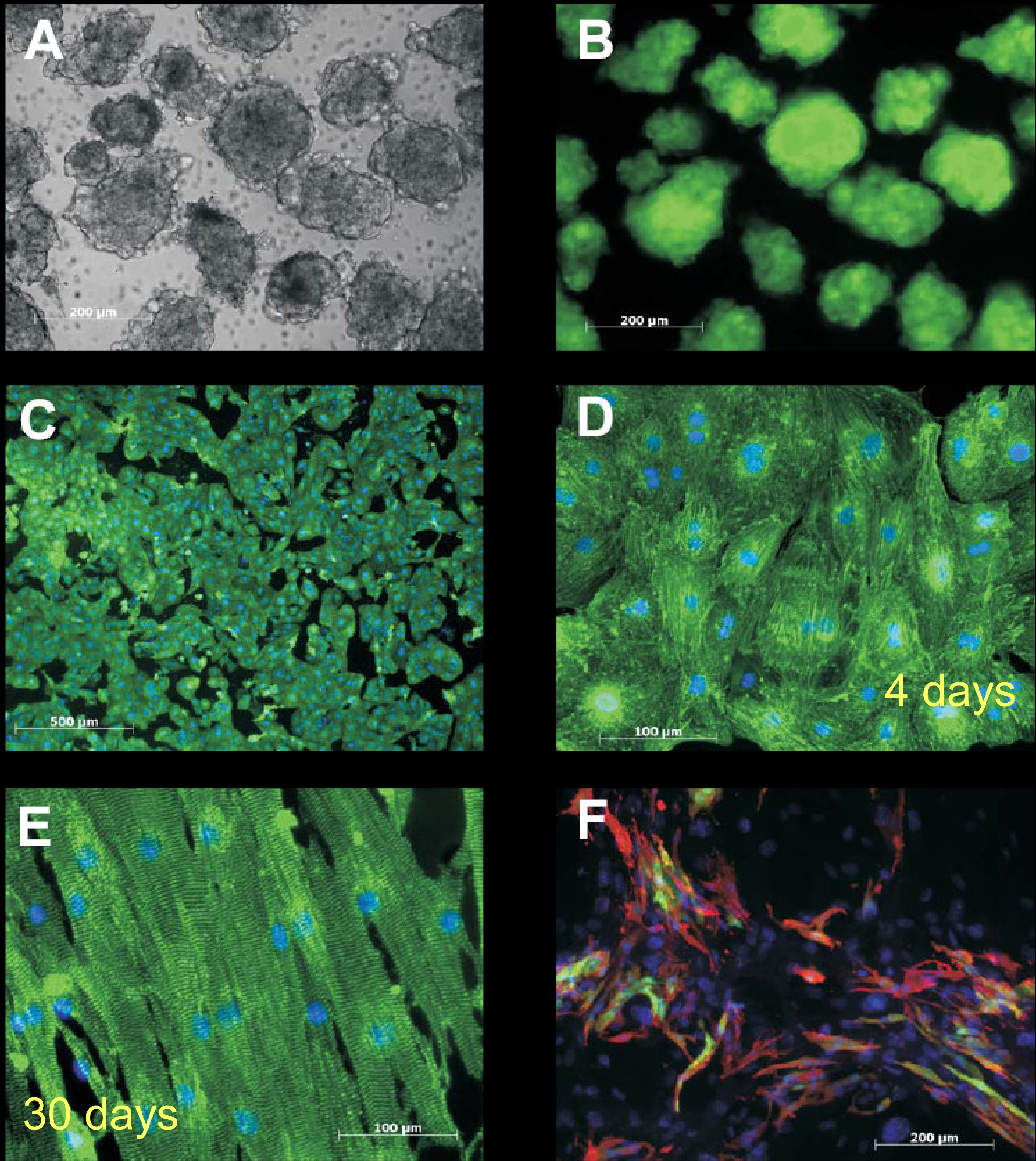
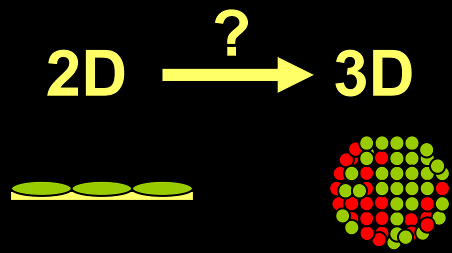
Attachment of cardiomyocytes to different polymer surfaces (from 3D to 2D)

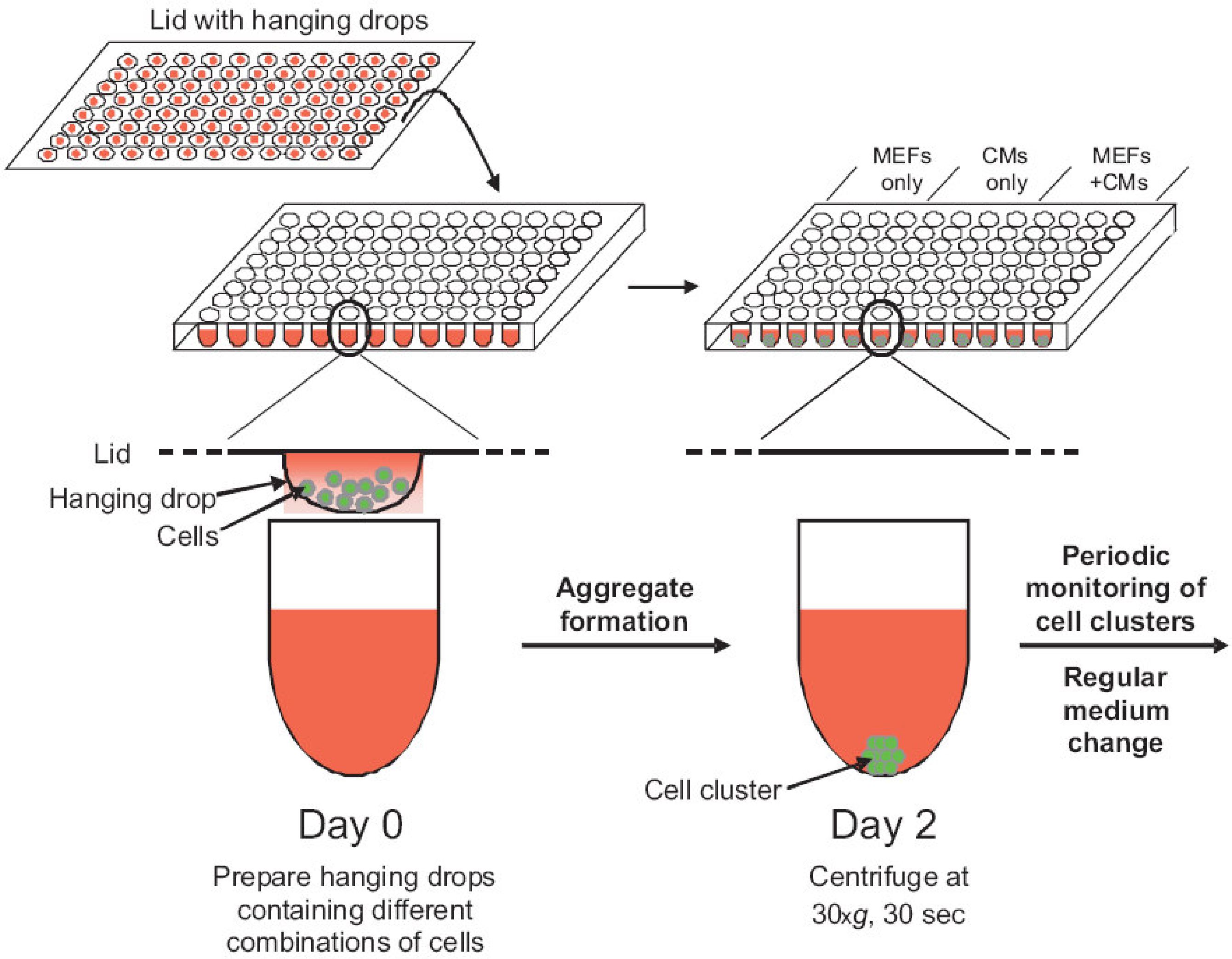




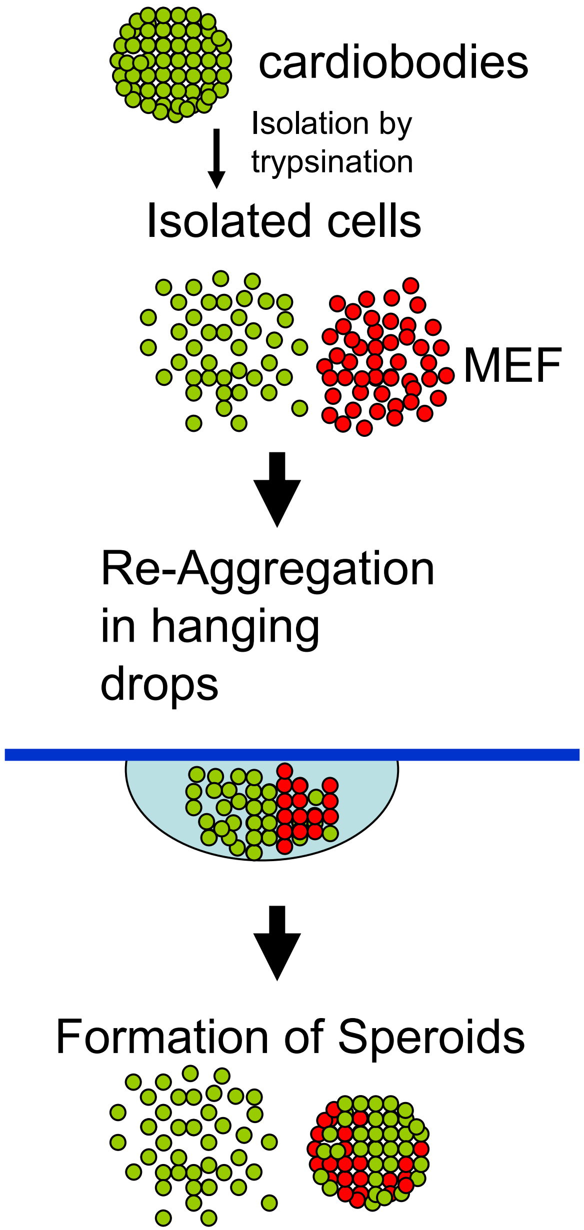
Microscopic assessment of cardiomyocytes purity and survival in short and long term 2D cultures plated on <u>fibronectin</u>-coated surfaces

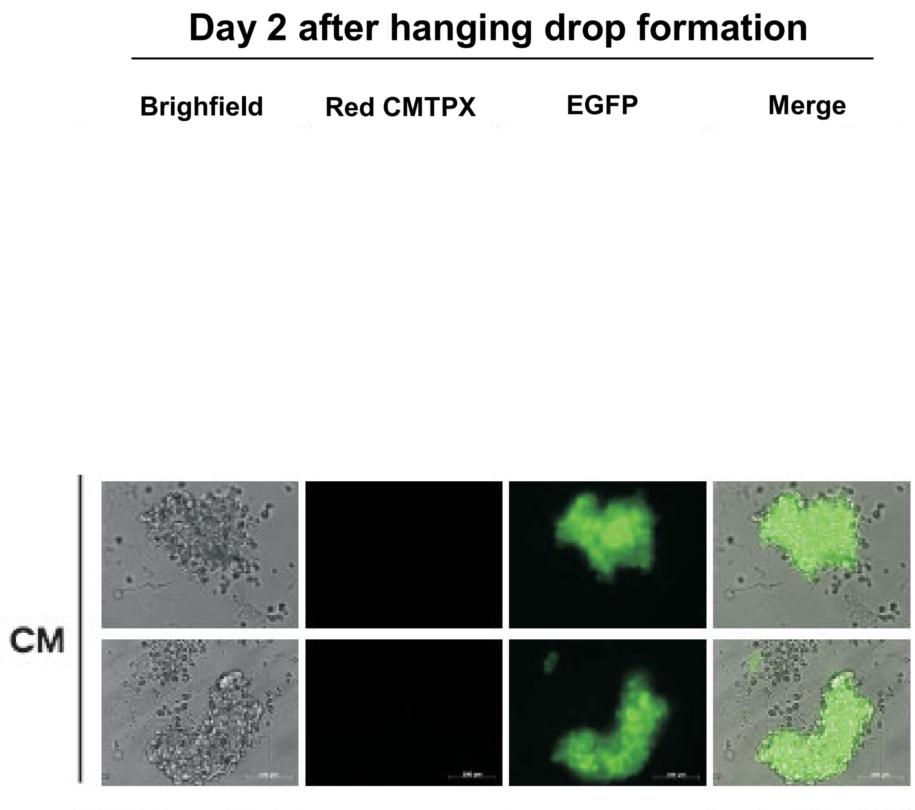


Schematic of cell aggregation using hanging drop method

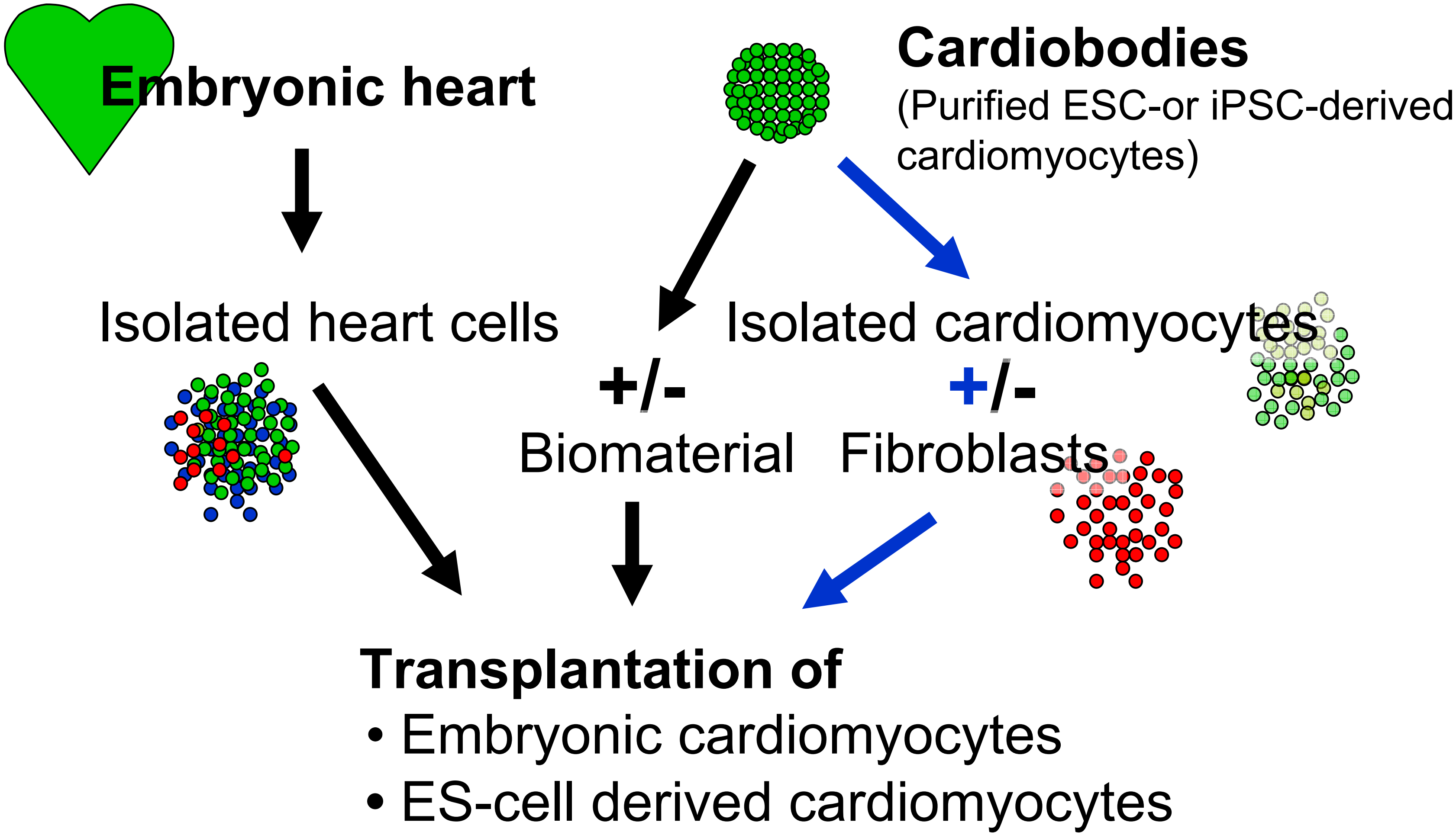


MEFs support reaggregation of single cardiomyocytes in 3D cultures within hanging drops (from 2D to 3D)

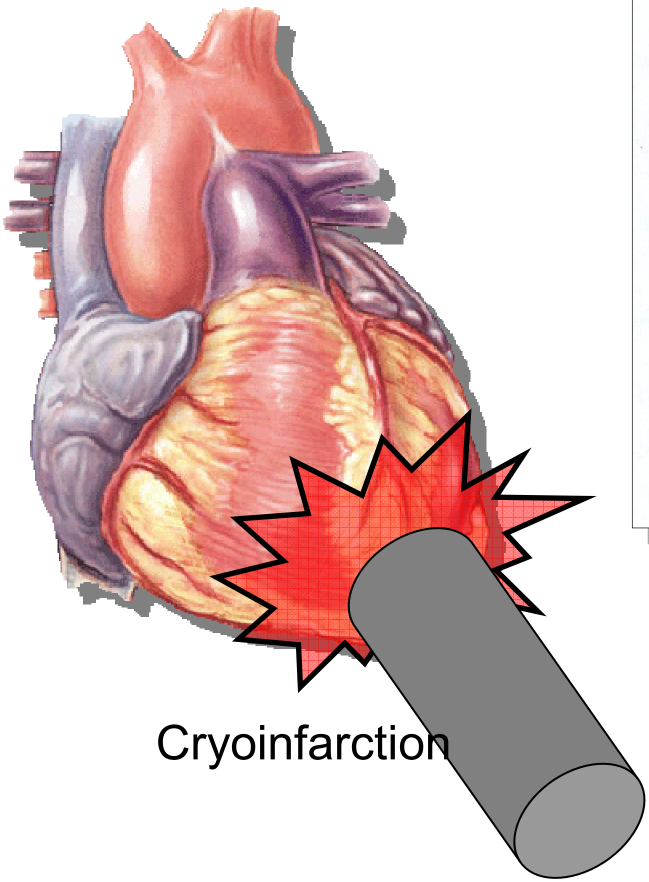


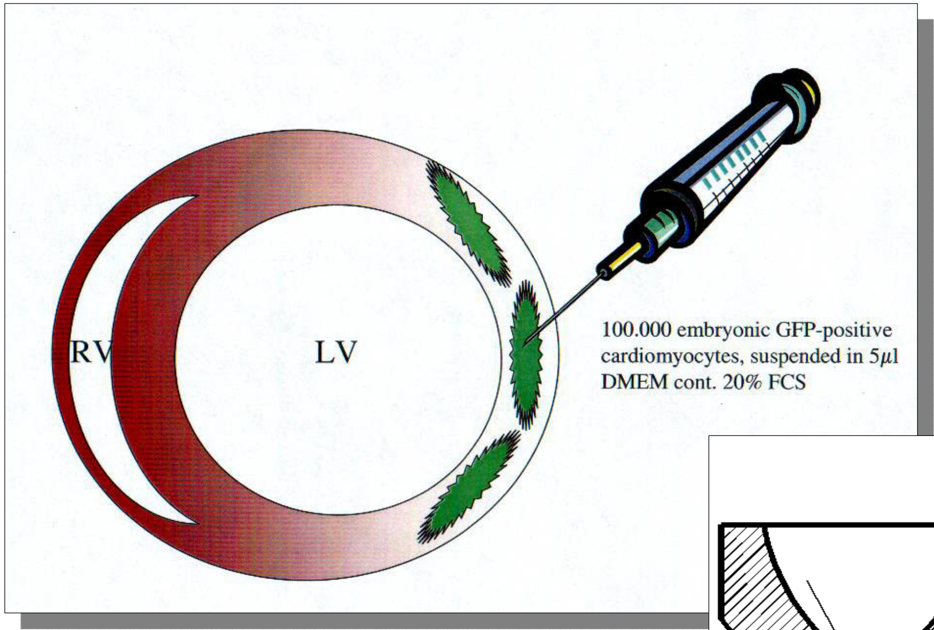


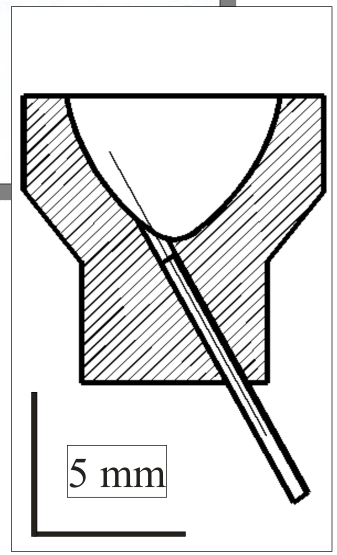
Cellular Cardiomyoplasty



Transplantation model



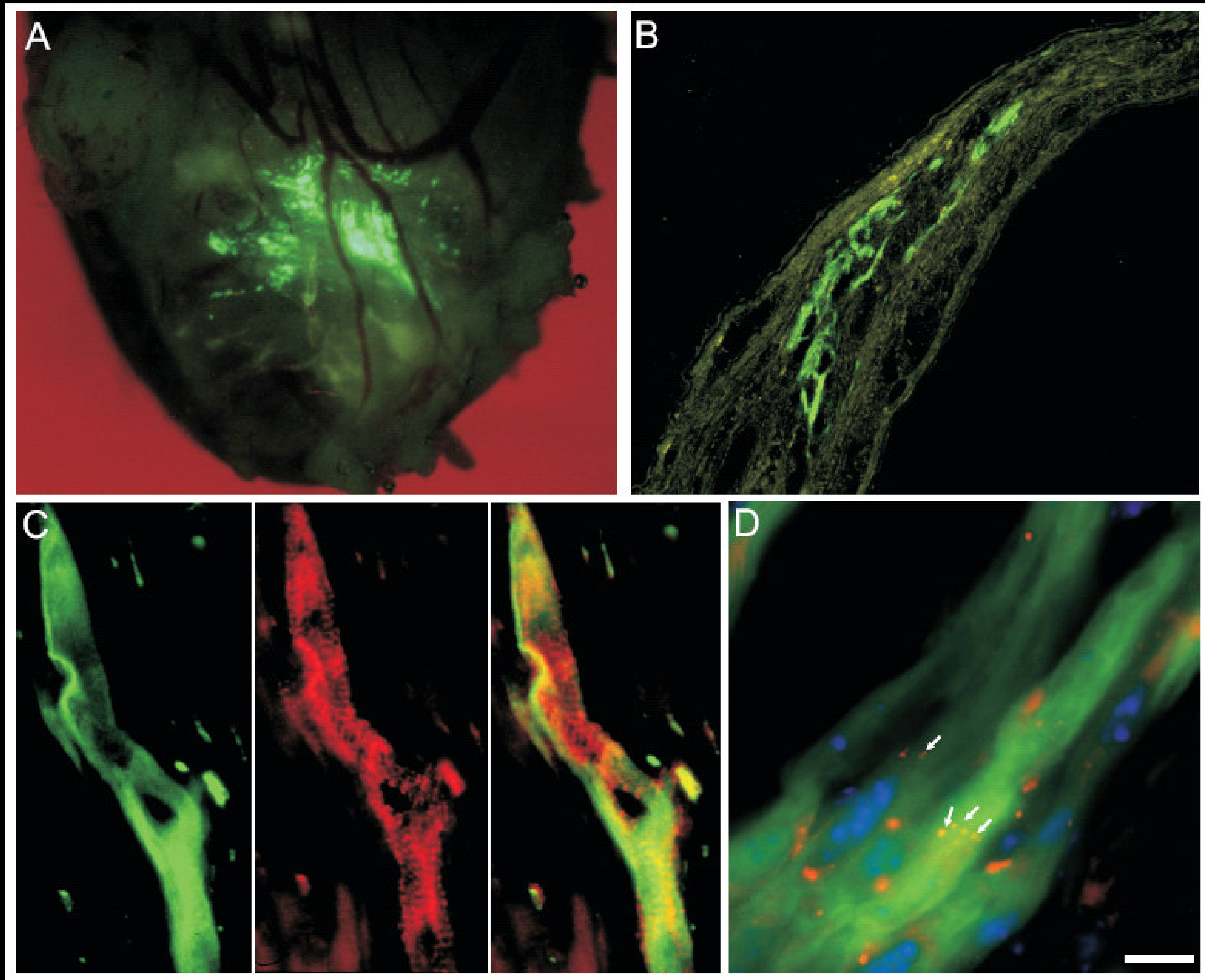




scheme

Roell et al, Transplantation 2003
Roell et al, Circulation 2003

Embryonic stem cell derived cardiac precursor cells after transplantation



Establishment of Slices from Murine Heart Ventricles

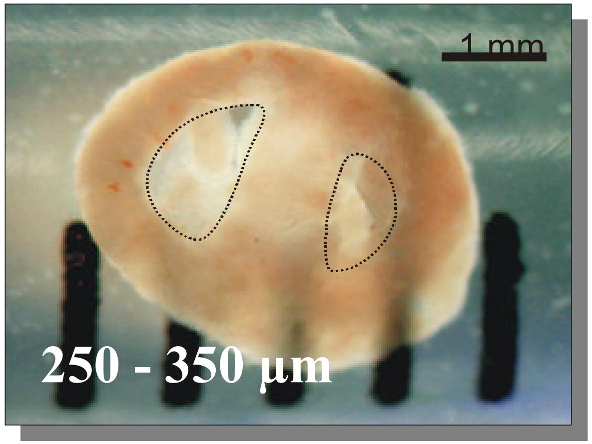
to study the functional Integration of Cardiomyocytes into Cardiac Tissue

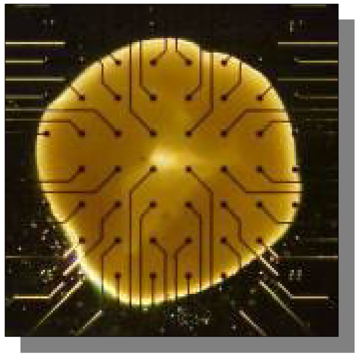
Heart → **Transplantation of purified cardiomyocytes** —1-20 days→ **Slices** → **Measurement of integration (*in vivo*)**

Slices → **Attachment and integration of purified cardiomyocytes in functional cardiac tissue** → **Measurement of integration (*in vitro*)**

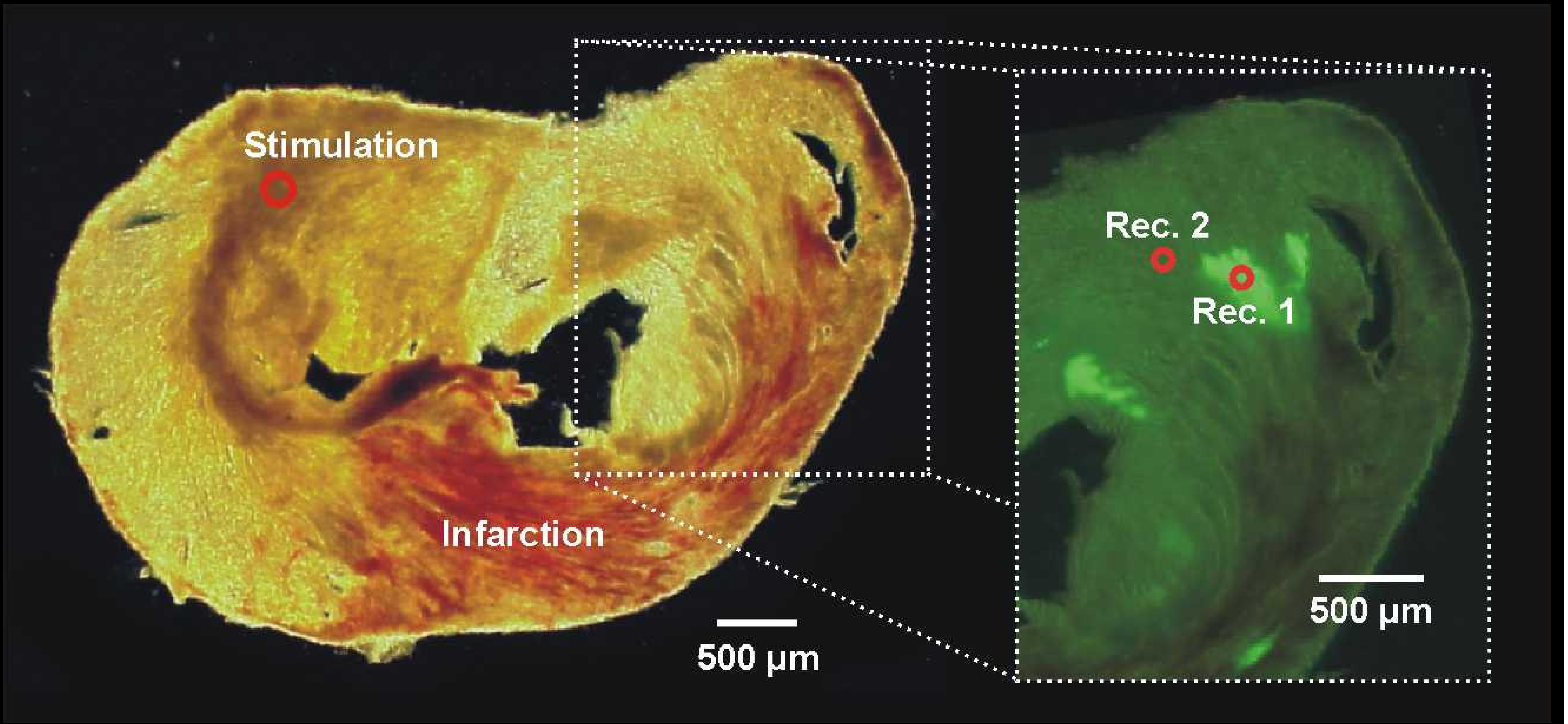
Hypoxic treatment of slices → **Attachment and integration of purified cardiomyocytes in cardiac matrix** → **Reconstitution of force of contraction**

Matrix substrates → **Attachment and integration of purified cardiomyocytes in bioengineered matrices** → **Reconstitution of structure**



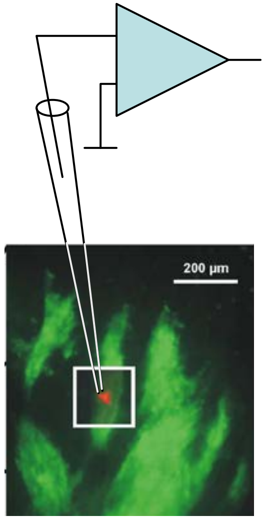


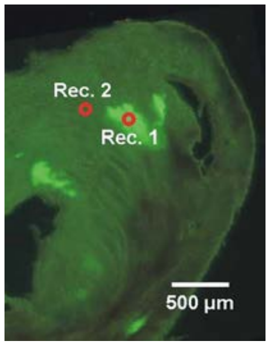
Establishment of Slices from Murine Heart Ventricles

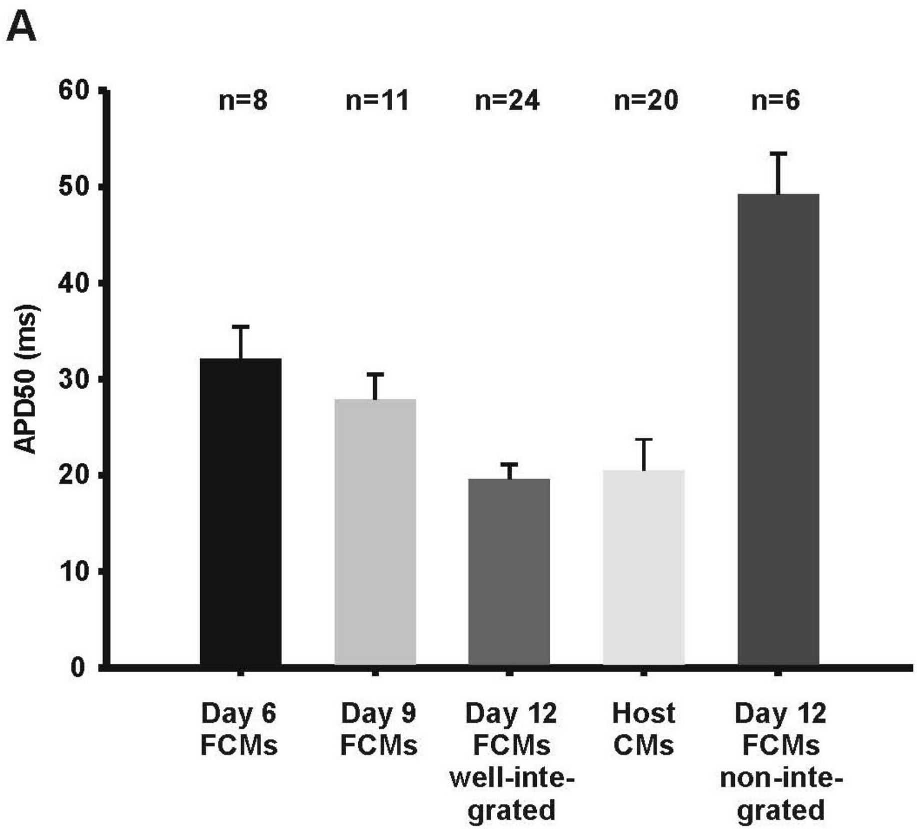


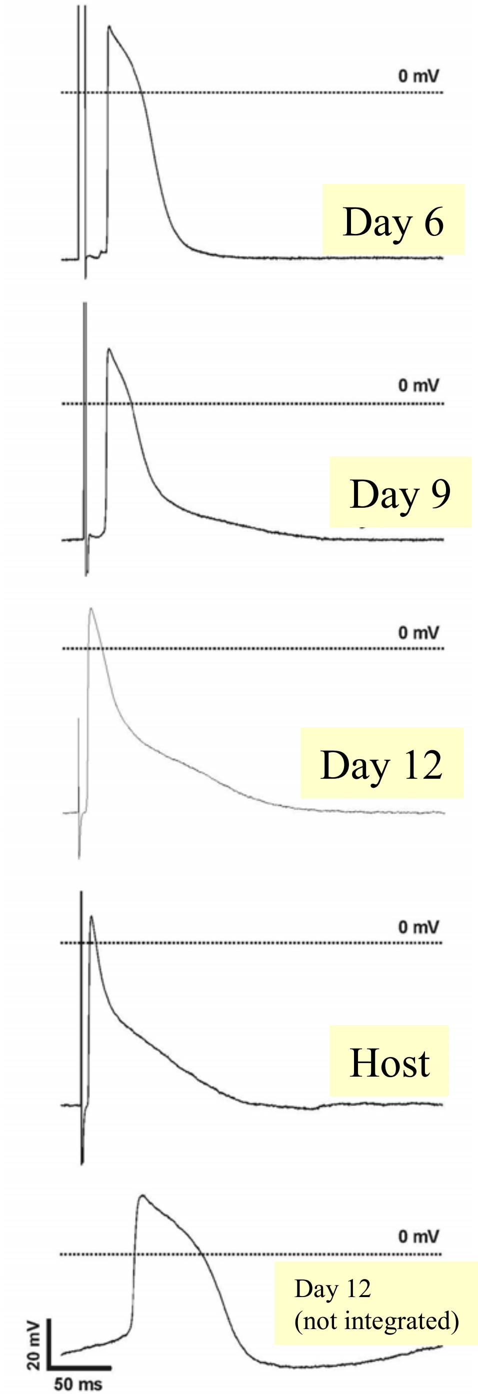
Maturation is a time-dependent process

(6 - 12 days after implantation)





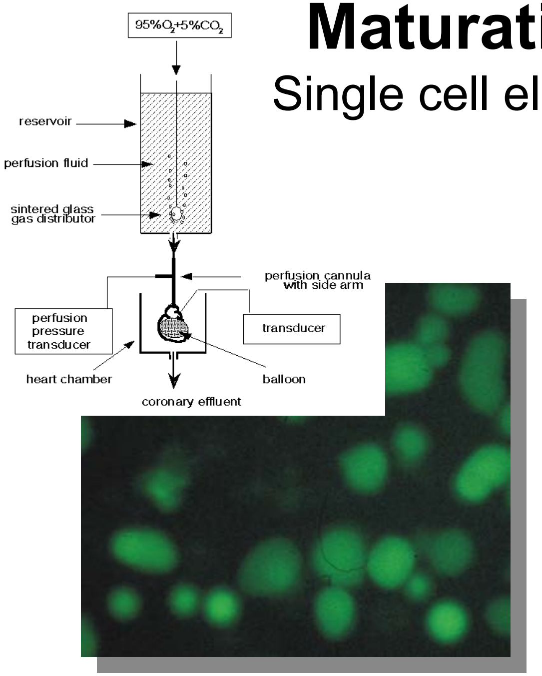


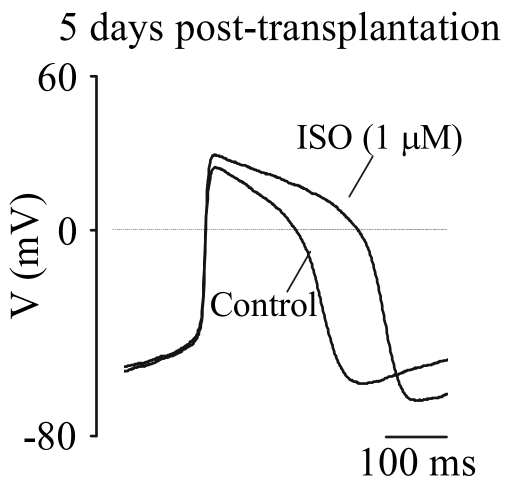


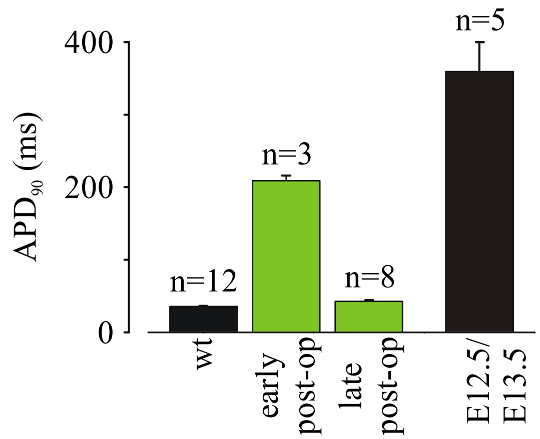
Q

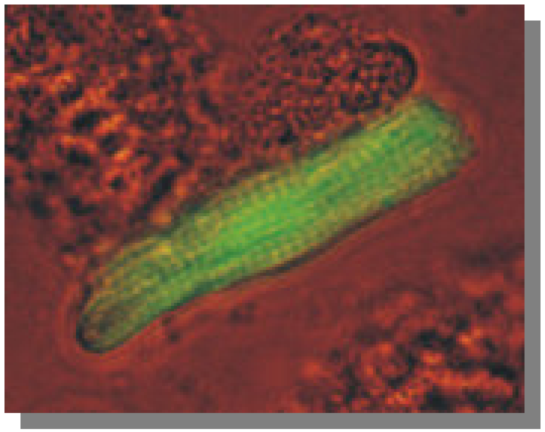
Maturation of CMs is a time-dependent process:

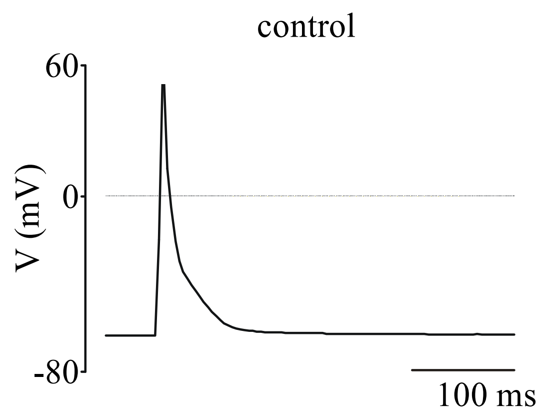
Single cell electrophysiology (cells were isolated by collagenase/trypsin)

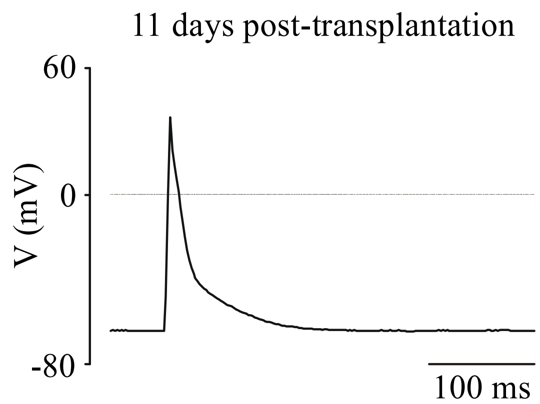






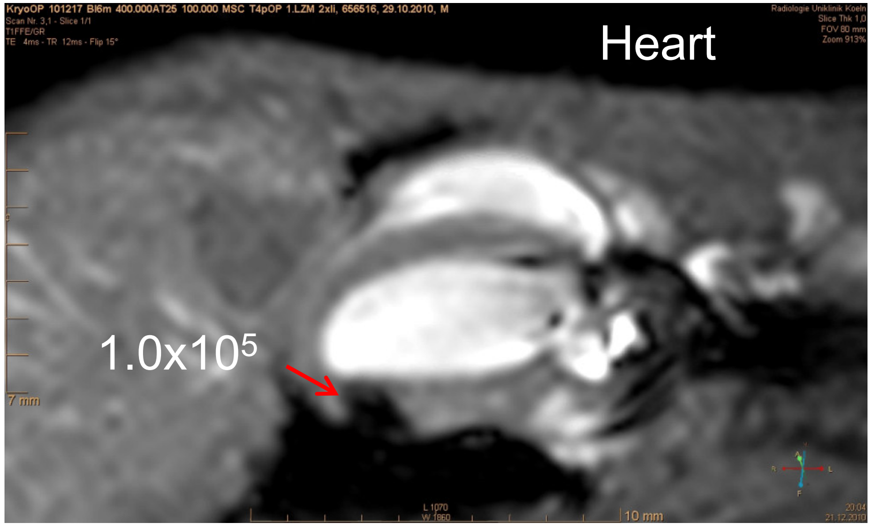




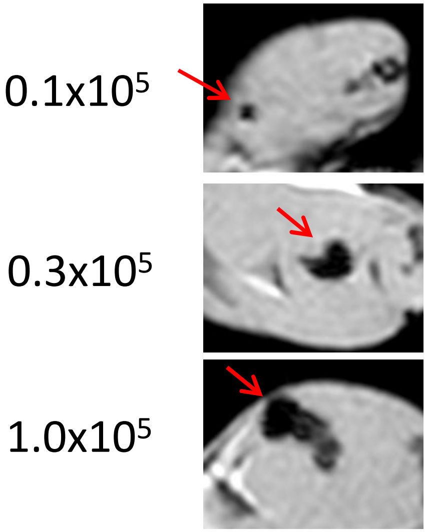


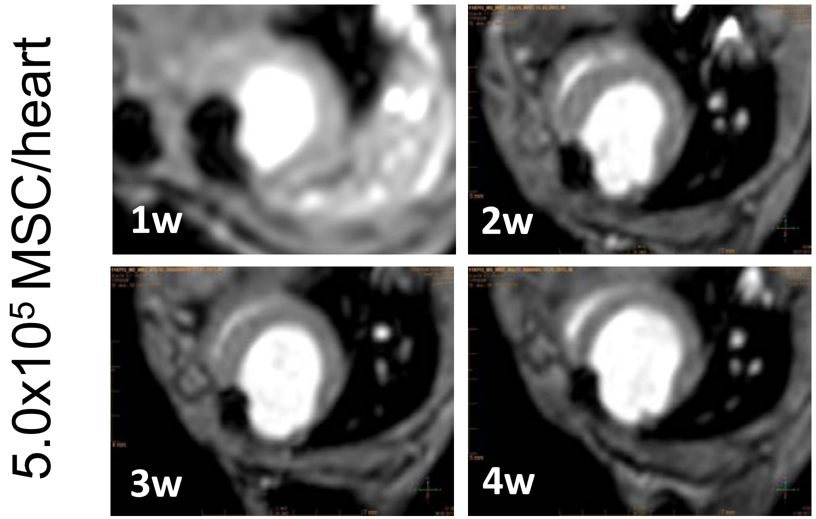
Bundesministerium
für Bildung
und Forschung

In vivo tracking of MSC by MRI



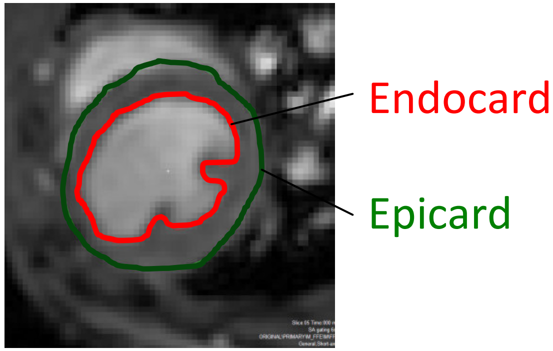
Skeletal muscle

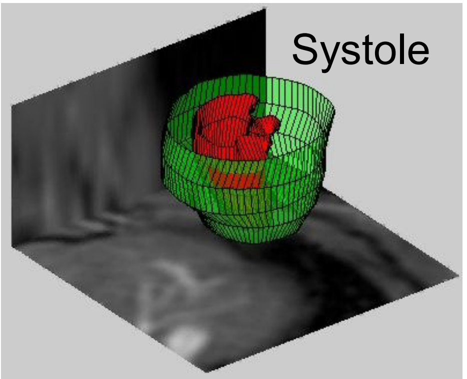


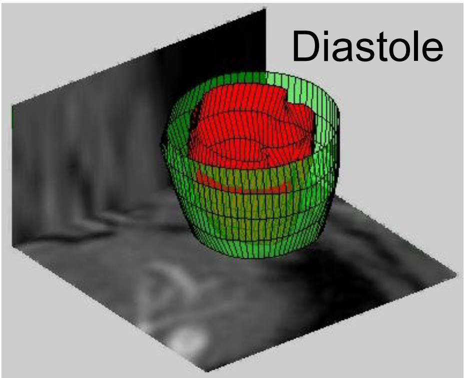


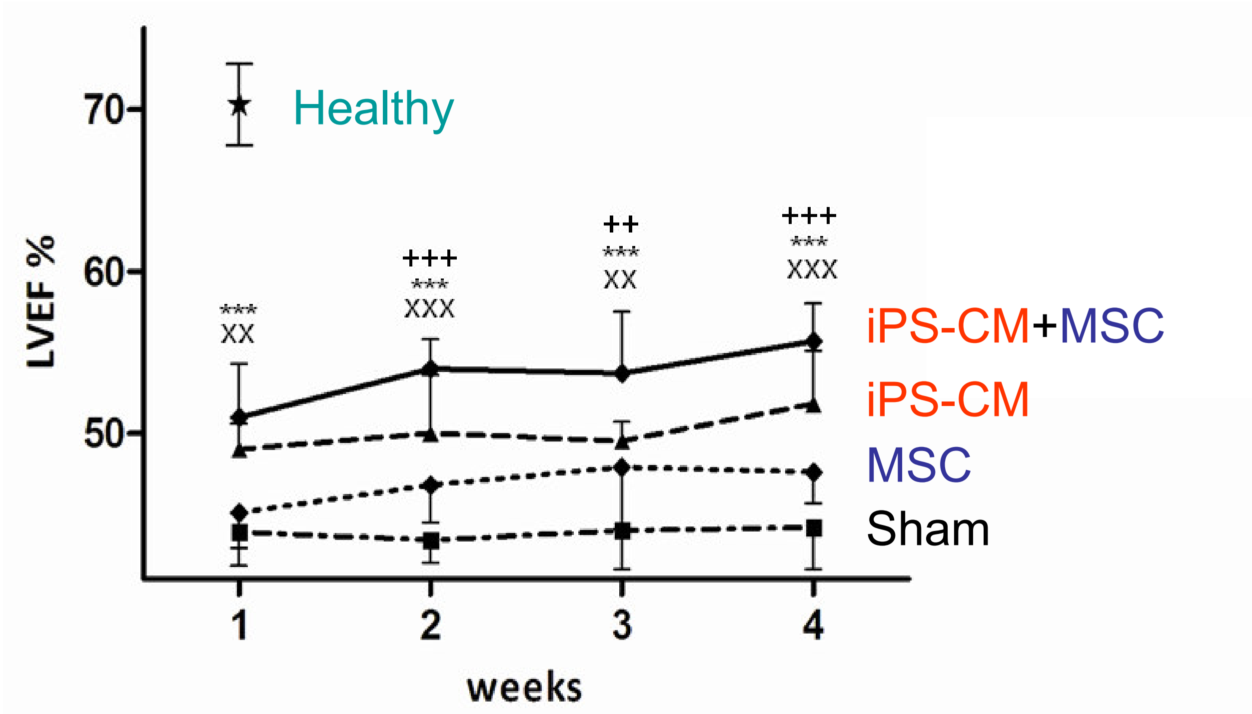
Florian Drey, Laboratory for cardiac regeneration (Neef/Choi group, Cologne)

Assessment of cardiac function by MRI









Bonferroni Multiple Comparisons Test:
x = MSC vs. CM+MSC
* = sham vs. CM+MSC
+ = sham vs. CM
p<0.001 (xxx/***/+++)
p<0.01 (xx/++)

Collaboration: Florian Drey, Laboratory for cardiac regeneration (Neef/Choi group, Cologne)


Stem Cell
Therapy
of Heart

Therapy based on pluripotent stem cells:
Proofs of Principle, Strategies and future improvements

In vitro

Human ES, iPS cells:
Prevent contamination
Differentiation, Mass culture √

Establish pure cultures of early organotypic cells
„lineage selection“ √

Test physiological function √

In vivo

Demonstrate efficacy:
Integration into host, Physiology, √

Demonstrate safety (tumor formation, √ contamina-tion) Tumors other than teratomas

Test methody to prevent rejection
Reprogramming, hiPS cells, cell bank, √

Preclinical Experiments

Large animal experiments

Good Manufactor-ing Practise (GMP)

Standart Operation Procedurs

Human trials

Quality Control

Pluripotent Stem Cells Go to Clinic

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Yamanaka: iPSCs Could Help Fill Our Blood Banks

By Juliana Chan | Editorials
October 13, 2014

Imagine a world where we no longer need blood donors, says Professor Shinya Yamanaka, Nobel laureate and inventor of induced pluripotent stem cell technology.

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AsianScientist (Oct. 13, 2014) - Imagine a world where we no longer need blood donors. A world where blood banks are filled to the brim with every possible blood type, coaxed from stem cells that originated from our skin. A world where artificial red blood cells (erythrocytes) are routinely used for blood tranfusions.

Such is the dream of Professor Shinya Yamanaka, Director of the Center for iPS Cell

theguardian

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News > Science > Stem cells

Stem cell therapy success in treatment of sight loss from macular degeneration

After three years of follow up, researchers say the treatment is safe and most patients have notably improved vision

Ian Sample
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The Guardian, Wednesday 15 October 2014
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This UCL photo shows an eye diseased with age-related macular degeneration, the leading cause of blindness among the elderly in the developed world. Photograph: UCL/PA

Lancet, October 2014

Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies

Steven D Schwartz, Carl D Regillo, Byron L Lam, Dean Eliott, Philip J Rosenfeld, Ninel Z Gregori, Jean-Pierre Hubschman, Janet L Davis, Gad Heilwell, Marc Spirn, Joseph Maguire, Roger Gay, Jane Bateman, Rosaleen M Ostrick, Debra Morris, Matthew Vincent, Eddy Anglade, Lucian V Del Priore, Robert Lanza

Summary

Background Since they were first derived more than three decades ago, embryonic stem cells have been proposed as a source of replacement cells in regenerative medicine, but their plasticity and unlimited capacity for self-renewal raises concerns about their safety, including tumour formation ability, potential immune rejection, and the risk of differentiating into unwanted cell types. We report the medium-term to long-term safety of cells derived from human embryonic stem cells (hESC) transplanted into patients.

Methods In the USA, two prospective phase 1/2 studies were done to assess the primary endpoints safety and tolerability of subretinal transplantation of hESC-derived retinal pigment epithelium in nine patients with Stargardt's macular dystrophy (age >18 years) and nine with atrophic age-related macular degeneration (age >55 years). Three dose cohorts (50 000, 100 000, and 150 000 cells) were treated for each eye disorder. Transplanted patients were followed up for a median of 22 months by use of serial systemic, ophthalmic, and imaging examinations. The studies are registered with ClinicalTrials.gov, numbers NCT01345006 (Stargardt's macular dystrophy) and NCT01344993

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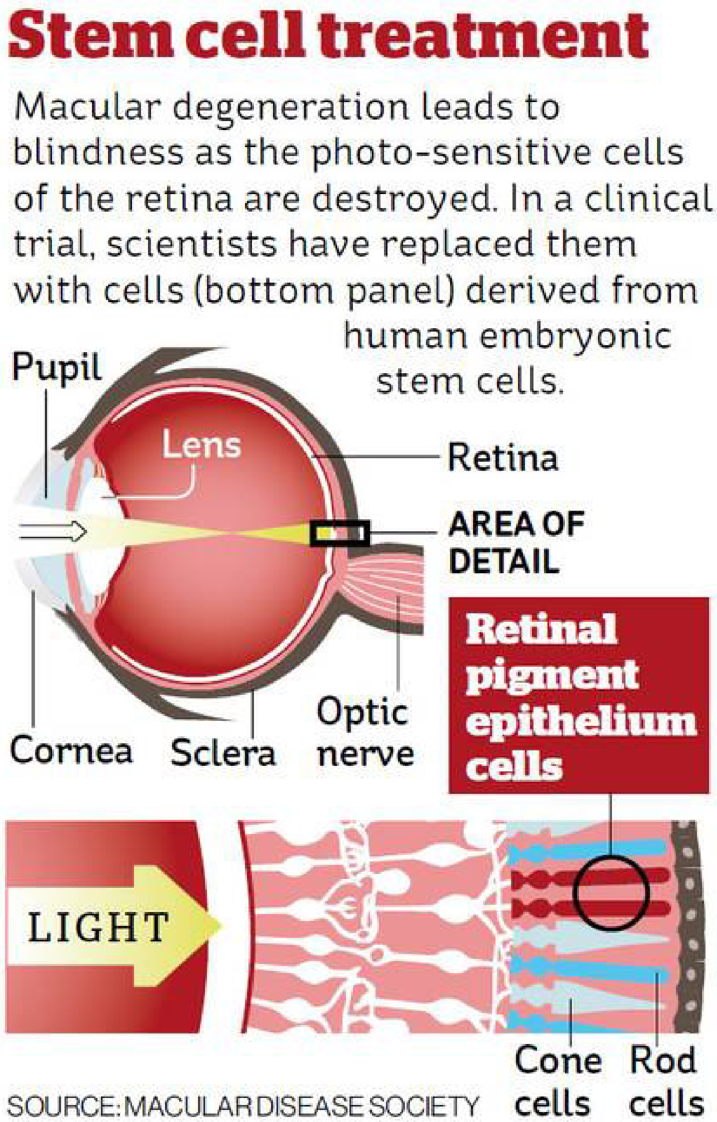
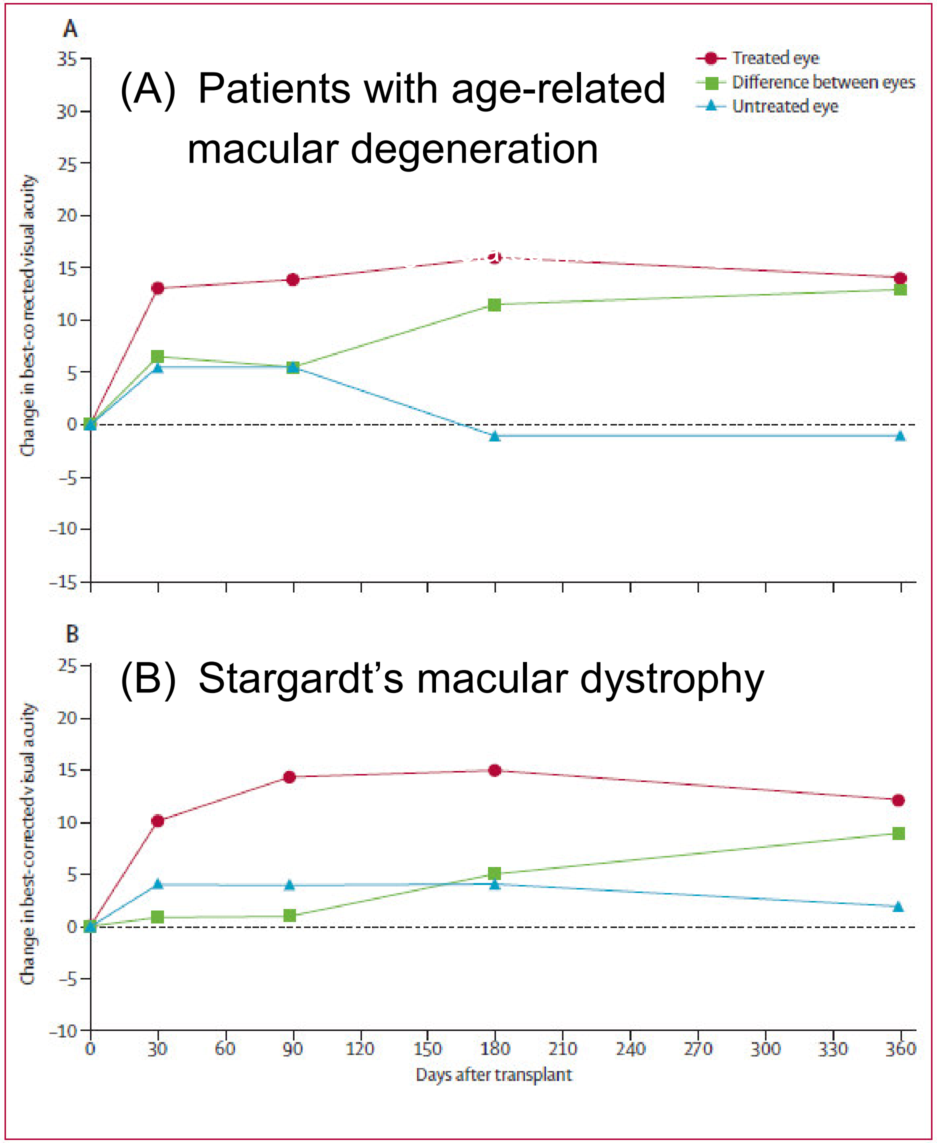
Jules Stein Eye Institute Retina Division, and David Geffen School of Medicine, University of California, Los Angeles, CA, USA (Prof S D Schwartz MD, J-P Hubschman MD, G Heilwell MD, R M Ostrick MPH);





Steven D Schwartz et al., Lancet 2014

Summary of Usage and Opportunities of Human Embryonic Stem Cells / iPS Cells

来自英国干细胞研究杂志的对于干细胞未来市场统计与预期分析

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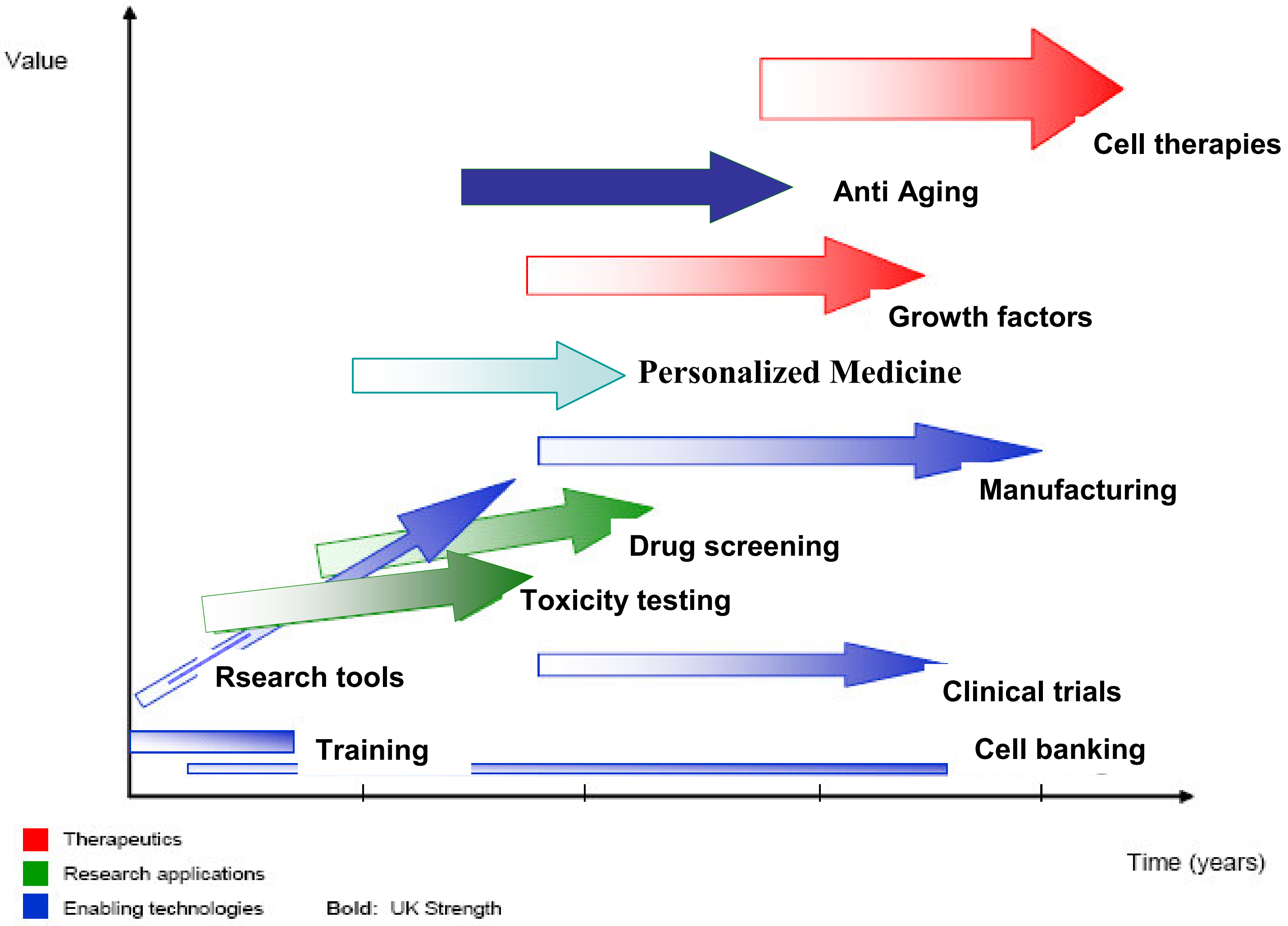

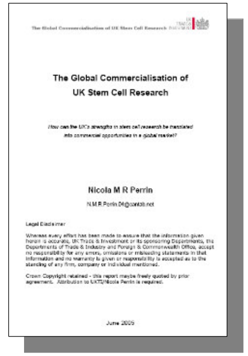

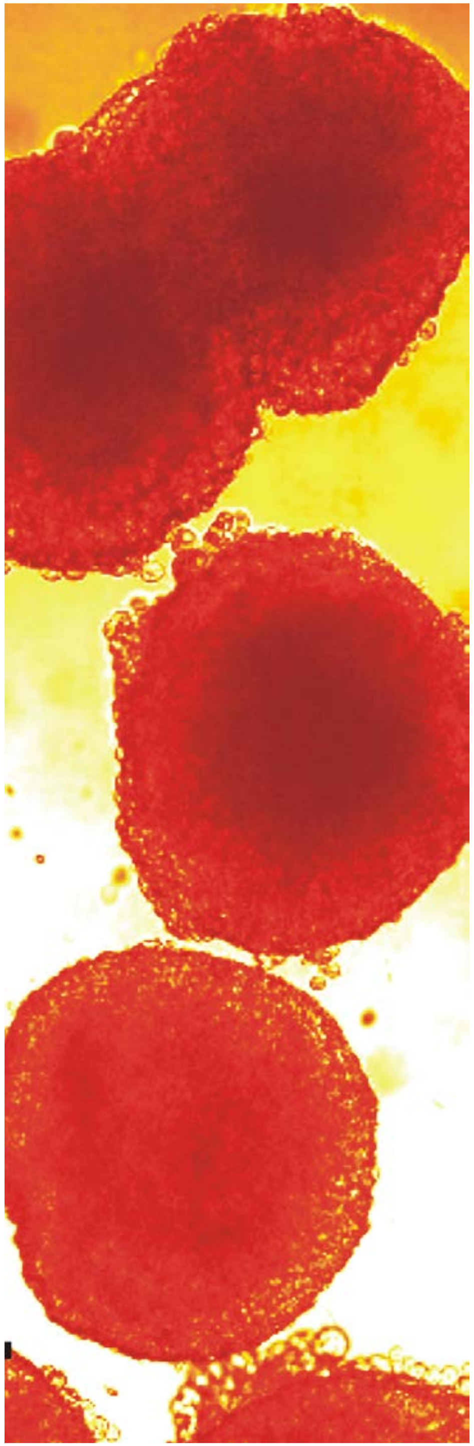

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MUSC Charleston	Mattin Morad Lars Cleemann

Greetings from our Cologne group
20th year jubilee

20 JAHRE JUBILÄUM

Greetings from our Cologne group
20th year jubilee


Thank you!